DIAGNOSTIC USEFULLNESS OF LEUKOCYTE ESTERASE DIPSTICK TEST FOR DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOTIC PATIENTS IN A TERTIARY CARE HOSPITAL.

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CERTIFICATE

This is to certify that this dissertation entitled **"DIAGNOSTIC USEFULLNESS OF LEUKOCYTE ESTERASE DIPSTICK TEST FOR DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOTIC PATIENTS IN A TERTIARY CARE HOSPITAL."**is the bonafide work done by **Dr.T S RAMESH KUMAR** in the Department of Stanley medical college, Govt. Stanley Medical College &Hospital, Chennai, in partial fulfillment of the regulation for **DM MEDICAL GASTROENTEROLOGY** examination of the Tamil Nadu Dr.M.G.R.Medical University, Chennai, to be held in AUGUST 2014.

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DECLARATION

I, **Dr.T S RAMESH KUMAR,** solemnly declare that this dissertation **““DIAGNOSTIC USEFULLNESS OF LEUKOCYTE ESTERASE DIPSTICK TEST FOR DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOTIC PATIENTS IN A TERTIARY CARE HOSPITAL.”** is the bonafide work done by me at the Department ofMedical gastroenterology, Government Stanley Medical College and Hospital,Chennai, under the guidance and supervision of**Prof.Dr.A.R.VENKATESWARAN,** Professor and Head of the Department ofMedical gastroenterology, Government Stanley Medical College, Chennai - 600 001. This dissertation is submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of **DM MEDICAL GASTROENTEROLOGY** examinations to be held in AUGUST 2014.

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ABBREVIATIONS

AFI - Ascitic Fluid Infections

DCLD - Decompensated Liver Disease

SBP - Spontaneous bacterial peritonitis

MNB - Monomicrobial nonneutrocytic bacterascites

CNNA - Culture Negative Neutrocytic Ascites

CNNNA - Culture Negative Non-Neutrocytic Ascites

PCR - Polymerase Chain Reaction

HRS - Hepatorenal Syndrome

CTP - CHILD TURCOTTE PUGH SCORE

HE - Hepatic Encephalopathy

MELD - MODEL FOR END- STAGE LIVER DISEASE SCORE

SAAG - Serum-ascites albumin gradient.

PMN - Polymorhonuclear Leukocytes

LERS - Leukocyte Esterase Reagent Strips

SID - Selective Intestinal Decontamination

MGIT - Mycobacterial Growth Indicator Tube

INTRODUCTION

Ascites refers to excessive pathologic accumulation of fluid in Peritoneal cavity[27]. The most common cause of ascites is CIRRHOSIS WITH PORTAL HYPERTENSION (85%) [14which] occurs in 50% within 10 years of diagnosing cirrhosis[40]

The development of ascites denotes the patient progresses to decompensated cirrhosis.Other complications are variceal hemorrhage, hepatic encephalopathy or jaundice; ascites is the most common[54]..

It is due to factors involving the peritoneum (malignancy), infection,or diseases away from the peritoneum , heart failure, hypoproteinaemia,liver disease).

Cirrhosis most important causative agent of ascites in the West (76%),peritoneal malignancy (14%), cardiac failure (5%) and peritoneal tuberculosis(4%).

Cirrhosis is a linguistic disorder with indolent course and many patients will be asymptomatic until decompensation. Early and well-compensated cirrhosis usually present as loss of apetite and weight loss, malaise, fatigue and weakness[28].

Decompensated Liver Disease is an end stage liver disorder with liver fibrosis and with complications like ascites, variceal bleeding,hepatic encephalopathy,enzymes production and reduced metabolism,hepatopulmonary syndrome [54].

DCLD patients have a poor prognosis and their 1-year and 5-year survival rates of 80% and 56% respectively[40].

Cirrhosis is an immunosuppressed status where both cell mediated and humoral immunity are decreased [21,54].These patients have reduced serum bactericidal opsonins, complements(C3,C4), protein C and fibronectin [39]. There is local and systemic immune dysfunction. Owing to porto-systemic shunting bacteria and bacterial endotoxins are not cleared by liver from portal circulation[21]. This predisposes patients to lot of infections [21]. This is later complicated by alcohol consumption, malnutrition, invasive procedures, catheters, immunomodulatory[21].

DCLD is associated with increased susceptibility to infections[22]. Bacterial infections takes for 37%-57% of deaths in these patients[21].

Nosocomial infections have an rate of 30%-32% and may be upto 49% with variceal bleeding[21].The most common bacterial infections inthe descending order of frequency are Spontaneous bacterial peritonitis (SBP) (15-30%),Urinary tract infections (25-29%), pneumonia (18-22%),bacteremia (17%) and soft tissue infection (15%).

Gastrointestinal variceal hemorrhage is accompanied with infection in60% of cirrhotics due to disruption of intestinal mucosal barrier andinvasive procedures during bleeding.

Infection triggers cytokine pathway with release of vasoactive mediators which causes increased variceal pressure, disordered homeostasis leading to furthervariceal bleeding.

Infections are most common if there is failure to control gastrointestinal bleeding within 120 hours and associated with earlyrebleeding rate .Antibiotic prophylaxis prevented infection and episodes ofrebleeding and also the need for blood transfusion[70].

The prevalence of this condition in those hospitalized with DCLD is 10-37% with 28% of those with hepatic encephalopathy[44]. The recurrencerate after first SBP episode is 41% at 5months,63% at 1year and 72% at two years[37] and sensible prophylaxis can reduce the recurrence rate to25%[3]. The frequency of ascitic fluid infection among outpatients is as lowas 0-3.7%[69].The in-patient mortality varies from 22-45%, with mortality rate of 57-72% at one year of follow up[44].

Serumcreatinine levels >1.5 mg/dl with lab culture were goodpredictors of mortality[44].The progress of underlying liver disease isdifficult to control and hence early detection and timely precisemanagement of infections can improve the outcome[70].

AFIs are under diagnosed by conventional culture methods since the median bacterial concentration is only approximately 1-2organismper milliliter[15].

Hence, this study was done to detect Ascitic Fluid Infections (AFIs) in outpatients & inpatients with Decompensated Liver Disease (DCLD), by bedside inoculation of ascitic fluid in leucocyte esterase ascitic fluid strip test. This could increase diagnosting infection at earlier time, facilitate rapid isolation of organisms and help to administer appropriate antimicrobialtherapy at the earliest.

AIMS & OBJECTIVES

1.The usefulness of leukocyte esterase ascitic fluid strip analysis in the rapid and early diagnosis of spontaneous bacterial peritonitis on decompensated chronic liver disease patients with ascites, so that rapid diagnosis of ascitic fluid infection and starting early treatment to reduce the morbidity and mortality of the disease which can be established.

2.The Objective is to find out the leukocyte esterase ascitic fluid strip analysis accuracy in the spontaneous bacterial peritonitis and after successful treatment with iv antibiotics the efficacy of the strip test in spontaneous bacterial peritonitis.

3.To study the Incidence and Prevalence of Ascitic Fluid Infections (AFIs) in Cirrhotic patients of various etiologies.

4. To Allegorize the prevalence of Ascitic Fluid Infections (AFIs) inoutpatients & inpatients with Cirrhosis.

5. To Establish whether bedside strip tests and bed side inoculation of ascitic fluid into bloodculture bottles combined with newer culture detection methods employing non-ionic surfactants (Triton-X 100 and Tween 80) helps in the identification of ascitic fluid infection.

6. To study the microbial spectrum of Ascitic Fluid Infections (AFIs) from outpatients & inpatients with DCLD.

REVIEW OF LITERATURE

Cirrhosis means a non remitting progressive, diffuse, fibrotic and nodular regeneration of liver so that the liver architecture is disrupted[61].

Long standing injury to the liver can proceed on to insult the liver to cause cirrhosis. There is persistent wound healing resulting in fibrosis.80-90% of the liver parenchyma must be destroyed for liver dysfunction to exhibit clinically[38].Cirrhosis is a indolent disease with silent course and many patients remain symptom free until they reachdecompensation.

Alcoholic liver disease(ALD) is an important risk factor for cirrhosis[38]. Individuals who consume large amounts of alcohol for prolongedperiod (about >64-84grams/day in males and>22grams/day in females over 11 years or longer) progress beyond the stage of steatosis of liver (92%) to develop alcoholic hepatitis and fibrosis (12-37%) then to cirrhosis (5-18%)[61.]

Women are more prone to develop Alcoholic liver disease within shorter life span due to increased activity of alcohol dehydrogenase activity in the gastric mucosa and liver and they have a lean body mass different from men and lower threshold toxic dose. This has been attributed to the gender
dependent differences in the hepatic metabolism of alcohol[61].

The complications of liver disease are due to impaired hepatic function and disruption of the normal hepatic architecture[61].

FLUID RETENTION IN CIRRHOSIS[40]

Ascites is a greek term (askes) which refers to a bag/sac[22].In cirrhotic patients there is more formation of vasoconstrictorswhich leads to increasedvascular tone. There is distortion of architecture of the liver. Both theselead to PORTAL (SINUSOIDAL) HYPERTENSION.This portal hypertension activates vasodilatory mechanisms. There is increased production of nitric oxide (NO) which leads to splanchnicand peripheral arterial vasodilation.This later causes decreased levelling of systemic vasculature and drop in pressure.This causes baroreceptor-inducedactivation of renin pathway with increased activity of sympathetic drive and arginine vasopressin .These events lead to renal sodium and water retention torestore normal homeostasis.Also,splanchnic vasodilation leads to increased lymph production and leakage into peritoneal cavity.Both the events lead to sustained ascites formation[40].

CLASSIFICATION OF ASCITES-THE INTERNATIONAL ASCITES CLUB[40]

Grade one Ultrasound detected.

Grade two Abdominal distension.

Grade three Tense Ascites

Ascites (Uncomplicated)Infection/HRS absent.

Refractory ascites prevention with drug treatment after therapeutic paracentesis.

Diuretic-resistant ascites high dose /maximum dose of diuretic treatment.

Diuretic-intractable ascitesdiureticscausing side effects leading to improper treatment.

DIFFERENTIAL DIAGNOSIS OF ASCITES[14]

Cirrhosis-85%

OTHERS-15%

Alcoholic hepatitis

Cancer (peritoneal carcinomatosis, massive liver metastases, etc)

"Mixed" ascites, i. e., cirrhosis plus another cause for ascites

Pancreatitis

Nephrotic syndrome

Tuberculous peritonitis

Heart failure

Acute liver failure

Budd-Chiari syndrome

Postoperative lymphatic leak

Sinusoidal obstruction syndrome

Myxedema.

HISTORY

It is of historical interest that Ludwig von Beethoven is probably the first patient known by name to have had SBP, especially since the clinical description of his case had been written 135 years before this syndrome was first described.*Kerr et al & Conn,* printed papers which explained ascitic fluid infections (AFIs) in the absence of contiguous or intra-abdominal source of infection[10].

In 1964, *CONN* was the one coined the term Spontaneous bacterial peritonitis (SBP)[3]. *Runyon* who has done several works in SBP suggests we now drop the term "SPONTANEOUS" since the pathogenesis has been studied and worked out[25].

PATHOGENESIS
GENERAL CONCEPT

In cirrhosis bacterial overgrowth leads to the migration from the intestinal lumen into mesenteric nodes and into the systemic circulation and other extra-intestinal sites since they pass through the liver and increased majority of portal flow diverts away from the liver sinusoids[41,42,21].This persistent bacteremia leads to ascitic fluid infection since it is an excellent bacterial culture medium[41,3.]

BACTERIAL OVERGROWTH

WHAT ARE THE POSSIBLE ROUTES BY WHICH BACTERIA MAY ENTER THE PERITONEUM?

Organisms can come directly from the gastrointestinal tract, from the blood stream.The rarest route is through the Fallopian tubes.This route of entry has been implicated by McCartney to explain the predominance of girls with primary peritonitis [(5).]

The most common causes of bacterial peritonitis were perforations of ulcers of the upper gastrointestinal tract or the rupture of abdominal viscera, usually the appendix. Although perforations of the gastrointestinal tract may be silent clinically, they are rarely so, and even when silent they usually exhibit pneumoperitoneum.Under certain conditions bacteria may enter the peritoneal cavity by traversing the intact intestinal wall[66].

Bacterial overgrowth occurs from the overgrowth of a single species of indigenous bacteria in the intestinal tract, immunosuppression, and thermal injury in which large segments of skin are burned, to hemorrhagic, hypotensive shock, i.e., insufficient blood supply to the gastrointestinal (GI) tract. In addition, specific disorders of the GI tract, such as intestinal or biliary obstruction or portal hypertension, may all give rise to Bacterial overgrowth[31].

It is possible that the hepatic lymphatics themselves may be involved in the pathogenesis of this syndrome. Hepatic lymph is

the key to the formation of ascites. In cirrhotic patients with hepatic venous outflow obstruction the production of hepatic lymph is increased resulting in the formation of ascites, due largely to the exudation of hepatic lymph directly into the peritoneal cavity[31].

II. WHAT ARE THE FACTORS THAT RENDER CIRRHOTIC PATIENTS PARTICULARLY PRONE TO DEVELOP SPONTANEOUS PERITONITIS?

Failure of hepatic removal of bacteria from the blood stream. McIndoe described the extrahepatic portal-systemic collateral networks that shunt portal venous blood around the liver.

Such portal-systemic shunts have been shown to diminish greatly thehepatic clearance of ammonia and other substances absorbed from the gastrointestinal tract.Presumably, these portal-systemic anastomoses permit circulating bacteria to bypass the hepatic reticuloendothelial filtering system, which has been shown to be the major site of removal of bacteria from the blood. Decreased hepatic removal of circulating bacteria tends to perpetuate bacteremia and thus afford circulating organisms a greater opportunity to cause metastatic infections at susceptible sites such as ascitic collections[41,42].

Normally the portal venous blood is aseptic. In case of migration of bacteria from infected lumen , they are getting trapped and removed by the liver. Cirrhotics have increased and abnormal bowel flora[41]. Bacterial overgrowth is increased in cirrhosis by delayed intestinal transit, decreased luminal IgA and bile salts[66].

Normal distal movements of luminal contents by peristalsis helps to avoid bacterial colonization and multiplication in the upper intestine. The complete or partial absence of the phase III activity of MMC(MIGRATORY MOTOR COMPLEX)-the "intestinal housekeeper" results in overgrowth[17]. In cirrhosis, there is increase in bacterial colonization of the small bowel (31-53%) with bacteria from the large bowel[38].

INTESTINAL MUCOSAL BARRIER
SECRETORY (1st DEFENCE) MECHANISM

The intestinal goblet epithelial cells secrets mucins thatelectro-negative charged layer prevent direct contact between bacteria and intestinal membrane. In cirrhotics there is elevated permeability of mucosa (patients withsepsis) due to oxidative stress, elevated Nitric Oxide and, endotoxemia, proinflammatory cytokine,enterocyte mitochondria malfunction [38].

IMMUNOGLOBULIN A

70% of body'simmunoglobulin production is IgA.Incirrhotics there is diminished mucosal IgA[17,38.12.]

BILE's TROPHIC EFFECT

Bile inhibits intestinal bacterial overgrowth; bile has detergent action and anti-adherence properties, endotoxin removal, trophic effect

for intestinal mucosa with decreased epithelial bacteria internalisation. The quantity of bile acids in liver disease is diminished due to decreased secretion and accentuated deconjugation of intestinal flora. It aids bacterial translocation caudes by endotoxins[17,38.]

THE PHYSICAL(2nd line of DEFENCE) MECHANISM

INTESTINAL EPITHELIAL STRUCTURE

Tight junctions between the cells located at the apicolateral surface of the epithelium inhibits bacterial or liopolysacchride transport. In liver disease there is intercellular spaces widening, diminished crypts and villus,Vasodilation,muscularis mucosae involovement,Oedema,and fibromuscular proliferation all dislodge the integrity of the normal epithelium[38].

NATURAL ANTIBIOTICS SECRETION

Paneth cells in the jejunal crypts and ileal crypts produce - phospholipase A2. defensins, and lysozyme,cryptidin related signal peptides,Angiogenin [4].

Small intestine epithelial cells and Colonic epithelial cells secrete – defensins that defend against commensal bacteria. In chronic liver disease secretion of these substances with antimicrobial activity is reduced.[13]

GUT ASSOCIATED LYMPHOID TISSUE.

Four components
Lymphocytes from the lamina propria.
Intraepithelial lymphocytes
Mesentric lymph nodes(MLN)
Peyer's patches

When the Bacteria interact with the structures in the Gut associated Tlymphocytes there is multiplication of lymphocytes, germinal center appearin the follicles and mucosal immunoglobulin secretion elevated [38].

The primary immune response was associated with monocytes, By its interaction of with *PRR-PATTERN RECOGNITION RECEPTOR with specific bacterial ligands PAMPs- PATHOGEN ASSOCIATED MOLECULAR PATTERNS.* Antigens are taken up by the dendritic cells through the local antigenpresenting cells (APCs)-*DIRECT MECHANISM* and by M cells whichovertake antigen by endocytosis-*IN DIRECT MECHANISM* [38].

Microbial peptides are presented by the Antigen presenting cells to the B & T lymphocytes. This will reach a climax in secretion of mucosal IgA(or IgG) or transformation into Th1 and Th2 cells. On exposure to the antigen, the T lymphocyte from the Peyer's patches[14]move towards lamina propria and getstranformed into CD8

cytotoxic T lymphocytes. Impaired primary and adaptive immune response occurs in DCLD[38,17.]

BACTERIAL TRANSLOCATION

Portal hypertension causes venous stasis, hypoxia of the mucosa and oxidative stress damage[28,30]. This leads to splanchnic dilatation with Mucosal congestion and Bowel Oedema leading to alteredpermeability and bacterial translocation to mesentric lymph nodes[40]. Gram negative bacteria are more adapt than grampositives and anaerobes in translocation[66].Bacteria generally do not migrate directly fromthe lumen into ascitic fluid. It happens if there is loss ofmucosal integrity. Anaerobes are present in excess in gut flora which translocate only in intestinal mechanical injury and are occasionally isolated from ascitic fluid in SBP[66,17]. More virulent organisms and Escherichia coli strains with greater adherence to intestinal mucosa translocate more precisely [21.]

INVASION OF ASCITIC FLUID AND BACTEREMIA

Splanchnic and systemic vascular dilatation with the formation of hyperdynamic circulatory state with elevated cardiac output and drop in blood pressure[21]. Bacteremia leads to spill over of the organisms from mesenteric lymph nodes into systemic circulation.[15]

Due to the diminished phagocytosis of reticuloendothelial system, the bacteria stays uncleared from the circulation[66]. In cirrhotics with intra and extra hepatic shunts the bacteria never come into alignment with the Kupffer cells and the bacteremia

increases[17].Bacteremia leads to colonisation of ascitic fluid as the infected fluid oozes off of the Glisson's capsule or when infected interstitial fluid oozes from the intestinal capillary bed.

SBP AND BACTERASCITES

Opsonins level and C3 complement are decreased in DCLD. Low protein concentration (<1g/dl) with positive correlation and decreased opsonic activity.Opsonins and macrophage were not able to kill the bacteria and so neutrophils are allowed to do the killing. SBP occurs, since there is impaired neutrophil function or qualitative neutrophil abnormalities.[40,58,66,17.]

SEPSISANDSYSTEMIC INFLAMMATORY RESPONSE SYNDROME

Molecular evidence of bacterial translocation is given by Identifying the DNA of the bacteria in the body fluid[47]. Lipopolysaccharides and peptidoglycans in the bacteria can trigger the TLR receptors[16]that leads to the production of pro-inflammatory cytokines. This leads to increased release of Tumor Cytokines that leads to inadequate tissue perfusion, multi- organfailure and death, refractory hypotension,sepsis syndrome pathways [21,42,58.]

ASCITIC FLUID INFECTIONS(AFI).

Most frequent (31%) of all infections among cirrhosis with portal hypertension[25]. Ascitic fluid infections(AFIs) has been classified into five variants based on analysis of the following parameters-

Polymorphonuclear leukocyte (PMN) count, culture growth and mode of entry of organism into the fluid[66].

ASCITIC FLUID INFECTIONS VARIANTS

VARIANTSOFASCITIC FLUID INFECTIONS(AFIs)	PMNCOUNT Cells/mm^3	CULTURE GROWTH
Spontaneous bacterialperitonitis(SBP)	•250	Monomicrobial
Culture-negative neutrocyticascites (CNNA)	•250	Nogrowth
Monomicrobial nonneutrocytic bacterascites (MNB)	<25	Monomicrobial
Secondary bacterial peritonitis-Indicates surgical cause such as perforated viscus/intra-abdominal abscess.	•250	Polymicrobial
Polymicrobialbacterascites-Perforation of the bowel by paracentesis needle.	<25	Polymicrobial

In symptomatic SBP caused by GPC, the PMN count is<250/$mm^{3(40)}$. SBP and CNNA variants of same disease and SBP was severe variant with greater mortality than CNNA[44. 17]

HEPATORENAL SYNDROME[4]

The annual incidence of HRS in patients with ascites is 10%.There is no major change in renal biopsy and is completely relieved by liver transplantation.

New Diagnostic criteria of the hepatorenal syndrome[12].

- Cirrhosis with ascites
- Serum creatinine >1.5 mg/dL (133μmol/L)
- No improvement of serum creatinine (decrease to a level of 1.5 mg/dL or less) after at least 2 days of diuretic withdrawal and volume expansion with albumin.
- The recommended dose of albumin is 1 g/kg of body weight per day up to a maximum of 100 g/day
- Absence of shock
- No current or recent treatment with nephrotoxic drugs
- Absence of parenchymal kidney disease as indicated by proteinuria of >500 mg/day, microhematuria (>50 red blood cells per high power field), and/or abnormal renal ultrasonography

  Adapted from Salerno et al. [12].

HRS TYPE 1

Renal failure with increased serum creatinine reaching more than 2.5 mg/dl[(12)] in <2 weeks. May occur spontaneously or in can be precipitated by bacterial infections, gastrointestinalhaemorrhage or acute hepatitis superimposed on cirrhosis. Develops in 30% of inpatients with SBP[32].

HRS TYPE 2

Moderate and steady decrease in renal function over months (serum creatinine < 2.5 mg/dl). There is severe ascites with poor or no response to diuretics (refractory ascites).

CHILD TURCOTTE PUGH (CTP) SCORE[61]

Initially used to prognosticate the short term mortality,it is now used to assess the prognosis and the requirement for liver transplantation. It contains parameterslike ascites and hepatic encephalopathy.

S.NO	VARIABLE	1POINT	2POINTS	3POINTS
1.	Totalbilirubin	<2mg/dl	2-3mg/dl	>3mg/dl
2.	Albumin	>3.5g/dl	2.8-3.5g/dl	<2.8g/dl
3.	INR	<1.7	1.7-2.2	>2.2
4.	Ascites	No	Mild (Medically controlled)	Severe (Poorly controlled)
5.	Hepatic Encephalopathy	No	GradeI-II (Medically controlled)	GradeIII-IV (Poorly controlled)

A : 5-6 points.B : 7-9 points. C : 10-15 points.

MODEL FOR END- STAGE LIVER DISEASE (MELD) SCORE[50]

Includes three lab objectives international normalized ratio(INR),serum creatinine and serumbilirubin. MELD=9.57×loge (creatinine)+3.78×log e(total bilirubin) +11.2×loge(INR) + 6.43.

MELD is better than CTP in evaluating the mortality following variceal bleeding. Addition of the creatinine which measures the renal function, explaining its importance in evaluation of mortality risk.CTP has more variability than MELD. Also MELD has wider possible scores and offers more weightage than CTP. Patients with high MELD score(>5) are more prone for infections and mortality than patients with low MELD score(<15).

PREDISPOSING AGENTS FOR ASCITIC FLUID CONTAMINATION IN DCLD[37]

- Severity of the liver disease-Child-Pugh class C patients
- Urinary tract infection and asymptomatic bacteruria[14]
- Serum total bilirubin level MORE THAN 2.5 mg/dl
- Gastrointestinal bleeding
- Low platelet count(<80,000/mm3)
- Increased prothrombin time[54]
- Increased liver enzymes[54]
- Previous episodes of SBP[21]
- Ascitic fluid protein level <1g/dl and complement C3 <13 mg/dl
- High creatinine and blood urea nitrogen

SIGNS AND SYMPTOMS

The most common reported are:

- Fever(68%)
- Abdominal pain(68%)
- Hepatic encephalopathy(53%)
- Ileus or Diarrhea(31%)
- Vomiting(20%)
- About 14% of patients with SBP are asymptomatic[37,63].

RECOMMENDATIONS FOR DIAGNOSIS OF AFIs.

The AFIs clinically presents with single or multiple symptoms. Considerable elapse of time leads to raised morbidity and mortality. The diagnosis is primarily on ascitic fluid analysis[5,40,3.]

Attempting to correct coagulopathy is not needed and is not cost Evading[3].

DIAGNOSTIC PARACENTESIS[3,5,37]

- New onset ascites
- Localising signs of peritonitis mentioned above[22]
- At the time of each admission to hospital
- In gastrointestinal bleeding prior to antibiotic prophylaxis.
- Any other laboratory test abnormalities
- Rapid impairement renal function
- Paracentesis is safe despite the predictable coagulopathy in cirrhotic patients.

❖ The risk of complication is negligible-prolonged leakage(m/c) 2% chance of abdominal wall haematoma, 0.02% chance of haemoperitoneum and 0.02% chance of iatrogenic infections (0.7%), visceral perforation. This clearly outways the benefits.[3,8,66.]

ASCITIC FLUID LABORATORY TESTS DATA[14]

ROUTINE	OPTIONAL	UNUSUAL	UNHELPFUL
Cellcount and differential	Culture in blood culture bottles	AFBsmear &culture	PH
Totalprotein	Gram'sstain	Cytology	Lactate
Albumin	Glucose	Triglyceride	Fibonectin
	Amylase	Bilirubin	Cholesterol
	Lactate dehydrogenase		Glycosaminoglycans

TESTS TO BE DONE FOR DIAGNOSIS OF AFIs[66]

Cell counts with differential

Culture (in blood culture bottles)

Gram's stain[23]

Total protein

Lactate dehydrogenase

Glucose

Amylase

Albumin (to calculate SAAG)

SAAG = Serum-ascites albumin gradient.

These tests also help to readily differentiate and identify the various etiologies of ascites besides from portal hypertension.

The SAAG (Serum-ascites albumin gradient) helps in delineating ascites better than the total protein based (transudate/exudates) criteria[14].

CORRECTED SAAG

In serum hyperglobulinemia (>5g/dl), narrow SAAG gradient occurs in 1% of ascitic fluid specimen.

CORRECTED SAAG = Uncorrected SAAG × 0.16 × (serum globulin [g/dl] + 2.5)

MIXED ASCITES[61]

Have underlying portal hypertension with cirrhosis along with other conditions like TB or peritoneal carcinomatosis. SAAG was more, due to underlying portal hypertension.[24]

MACROSCOPY: GROSS APPEARANCE61

- Crystal clear- protein level decreased
- Transparent and yellow-Non-neutrocytic ascites (PMN<250/mm3)
- Cloudy-Cells value of 5000/mm3 is cloudy,and greater than 50,000/mm3 appears mayonnaise.
- Bloody- Ascitic Fluid red blood of Ten thousand/mm3 is the maximum value.RBC more than 20,000/mm3 is always red.Seen in 50% of cases with cirrhosis and hepatocellular carcinoma.
- Chylous/Milky-AsciticFluidwith triglyceride content>200 mg/dl.

- Found in malignancy-related ascites and in about 21% of cirrhotic ascites.
- Dark brown-Biliary concentration more than that of serum, due to biliary perforation.

IMPORTANCE OF CELL COUNT

The ascitic fluid POLYMORHONUCLEAR LEUKOCYTES count (maximum> two fifty cells/mm3) is the efficient test for diagnosis of AFI.But PMN count above this value can also be found in bleeding into ascites,peritoneal carcinomatosis and pancreatic ascites [66].

In tuberculous or malignant ascites, lymphocytesincrease.

HAEMORRHAGIC ASCITES =CORRECTION FACTOR [5]

Accurate PMN count can be calculated only in non-traumatic tap.

Haemorrhagic fluid can occur in severe coagulopathy or in bloody tap, malignant ascites[5].

MANUAL VS AUTOMATED COUNTING

The laboratory should perform the cell count within 60 minutes. The manual count is error-prone and subjective and it also retards the start of ever needed empirical antibiotic therapy[3,13,69.]

Automated cell counters are ideal but the manufacturers do not recommend it for fluids other than blood[13].

This can be useful if further validated[14].

ASCITIC LEUKOCYTE ESTERASE REAGENT (LERS) STRIPS/ASCITIC FLUID NITRITE REAGENT STRIP DIPSTICS

Leukocyte esterase is an intracellular enzyme within the PMNs and is released upon lysis of PMNs during inflammatory cascade[20].The strip is placed inside a test tube of fresh ascitic fluid at the bedside and kept for 90 seconds.

The esterase present in the PMNs act on the ester substrate and releases 3-hydroxy-5-phenyl-pyrrole.this changes the color of an azodye in the reagent strip. The calorimetric scale reference chart is availablefor direct optical comparison on the sides of the container[3].

See for the purple colour on the strips which is equivalent to a PMN count of 250/mm3 so that first dose of empiric antibiotic can be given (reduces the tap-to-shot time). It isfaster and cheaper rapid. The sensitivity is 95%, specificity 88% and negative predictive value of 95%[13,69]. Example: *NEPHUR TEST AND MULTISTIXSG10*.However, study conducted on a large scale showed only 45% sensitivity[14].

SECONDARY PERITONITIS[40,5,20,54]

Look for it when:

1. No diminish in ascitic fluid PMN count 48 hours after antibiotic starting.
2. Two or more organisms shown on culture (espfungusor anaerobes).
3.If in ascitic fluid atleast two (2/3) is seen:

-- AF protein >1g/dl.

-- AF lactate dehydogenase(LDH)>225mU/ml(normal serumlevel).

-- AF glucose <50 mg/dl.

Antibiotics against anaerobes and enterococci have to be added.

RELAVENT ADDITIONAL INVESTIGATIONS[3,5]

1. Blood cultures associated with the ascitic fluid cultures-positive in atleast 1/3 rd of cases[26].
2. Complete blood count
3. WBC count may be low inspite of the presence of SBP due to Hypersplenism[57].
4. Urine analysis and urine culture-asymptomatic bacteriuria is an independent risk factor[14].
5. Liver function tests-Total and direct bilirubin, liver enzymes-AST, ALT, SAP, GGT, Total protein and albumin, globulin[54].
6. Cefotaxime 2g every 12 hr for 7 days is the common empiric antibiotic. Second ascitic tap done 48 hrs after start of therapy and there should be a 30% decrease in cell count. Otherwise change the antibiotic[3].

TUBERCULOUS PERITONITIS

Extrapulmonary disease *Abdominal tuberculosis* is the sixthmost frequent site[49].

PeritonealTB occurs in three types:

1. Fibrotic type
2. Encysted (loculated) type

3. Wet type with ascites

ASCITIC FLUID EXAMINATION

Macroscopically it is straw coloured and an exudates (protein >3g/L). The total cell count is 500-2000 cells/mm3 with predominant lymphocyte (70%). Lymphocytosis of ascitic fluid means they account for >30% of total AF cell count[39.]

In some PMNs are abundant(>250/mm3) early in the disease and this can lead to misdiagnosis as SBP[2,39,45.]

The SAAG has a gradient of <1.1g/dl. The adenosine deaminase (ADA) of >33U/L has a sensitivity of 98% and specificity of 100% in non-cirrhotic patients[4]. The yield of tubercle bacilli on smear and culture is low and large amounts of fluid(about 1L) has to be used for centrifuging and the deposit is inoculated on LJ medium. The time taken for growth is usually 6-8 weeks[2,29,45.]

CAUSES OF LOW GRADIENT (SAAG <1.1g/dl) ASCITES[14]

- TB-AF LDH (<250 U/ml) and glucose level lower
- Pancreatic ascites
- Malignancy induced ascites-AF LDH and glucose level higher
- Biliary ascites
- Renal ascites

SELECTIVE INTESTINAL DECONTAMINATION (SID)[23,36,37]

Norfloxacin is recommended :

Poor intestinal absorption and rapidly diffuse into ascitic fluid.

Preserves anaerobes and prevents gut colonization by pathogenic bacteria.

Strong activity against gram negative bacteria.

Has fewer side effects.

Should be started in[23]:

Patients with gastrointestinal bleeding ,Norfloxacin 400 mg BD × 7days *(SHORT TERM).*

AF protein level <1.5g/dl

Patients who have recovered from previous SBP episode. Newer quinolones are preferred since apart from eliminating gram negative bacteria,decrease bacterial adhesion to mucosa, and they also stimulate bactericidal capacity of PMNs.

Patients who survive an episode of SBP have 41 to 71% chance of relapse in the coming 13 months. Hence, long term administration of quinolones is advocated for these patients tillsymptoms and signs of ascites, transplantation or death. But this approach selects quinolone resistant GNB and trend towards infection caused by GPC. For patients on quinolone prophylaxis who develop anSBP episode, third generation cephalosporins are the bestoption[42].

MATERIALS AND METHODS

STUDY TYPE

Cross - Sectional Study.

STUDY PLACE

This study was done in Department of Medical gastroenterology, StanleyMedical College in association with the Department of Microbiology, Government Stanley hospital, Chennai.

STUDY PERIOD

November 2012 to JANUARY 2014.

STUDY POPULATION

During this period ascitic fluid from 422 cases of cirrhosis attending the Department ofMedical Gastroenterology, Govt. Stanley Hospital were examined forAscitic fluid Infections (AFIs).

Among the 422 DCLD patients, 141 were outpatients and 281 were inpatients. Among the 141 outpatients, 117 were men and 24 were women. Among the 281 inpatients,253 were men and 28 were women.

INCLUSION CRITERIA

- All inpatients with Decompensated Liver Disease (DCLD), beforethe first dose of antibiotic administration.
- All outpatients with recent onset undiagnosed ascites and
  Non repetitive out patients undergoing therapeutic paracentesis.

EXCLUSION CRITERIA

1. Patients who were on antibiotics in the previous two weeks.
2. Patients with secondary causes of peritonitis like gut perforation.

ETHICAL CONSIDERATION

Ethical with research clearance was obtained from the Ethical Committee Stanley Medical College. Written consent done before enrolment.

STATISTICAL DATA

Statistical data obtained with SPSS Software (Statistical Package for Social Sciences) version 20. Univariate analysis was doneusing Pearson Chi-Square Test and Fisher's Exact Test.

SAMPLE COLLECTION[61]

PREFERRED SITE FOR PARACENTESIS

Left lower quadrant was taken with two finger breadths cephalad and two finger breadths medial to the anterior superior iliac spine *(RUNYON'S SPOT)*[3], where the abdominal wall is thinner with larger pool of fluid. Right lower quadrant was not the choice since appendicectomy scar/dilated caecum are present.

The Inferior epigastric arteries and midline (collaterals) were Escaped. Visible collaterals were also escaped.

POSITION OF THE PATIENT

The head end of the bed elevated . Patients with large volume of ascites and thin abdominal wall were "tapped" in this supine position. Patients with less amount of fluid were placed in the lateral decubitus position and tapped from left lower quadrant.

NEEDLE OF CHOICE

Standard metal 1.5 inch,22 gauge needle is used, obese Individuals with thick abdominal wall use longer needle of 3.5 inch length .

DISINFECTION OF SKIN [6]

As per Universal precautions gloves that are sterile were worn for the procedure. The skin to be punctured is rubbed using 60% ethanol in a circle approximately 4.5cm in diameter and allowed to air dry. Starting at the center of the circle, 2% povidone iodine was applied, until the entire circle was saturated with iodine and allowed to dry for one minute.

TECHNIQUE OF PARACENTESIS

Under aseptic precautions the disinfected area was injected with local anaesthetic, punctured with 22 gauge needle using "Z tract" technique. The skin was displaced 2cm downward with one gloved hand and the needle was introduced. Needle was introduced through the abdominal wall gently. The syringe with the needle was aspirated during insertion.When the needle was released, the skin came back to its ground zero position. This helped the needle pathway to be sealed

and post-procedural leak was avoided. About 50 ml of ascitic fluid was withdrawn using syringe.

STRIP TESTING:

The fluid will be checked with a test tube containing the strip MISSION ASCITIC FLUID ANALYSIS REAGENT STRIPS 10 U.

The Strip test detects leukocyte esterasein azurophilic granules. A positive value usually shows bacterial presence.

The leukocyte esterase / NITRITE TEST test is helpful and we must not depend on this test to detect infection in ascitic fluid.

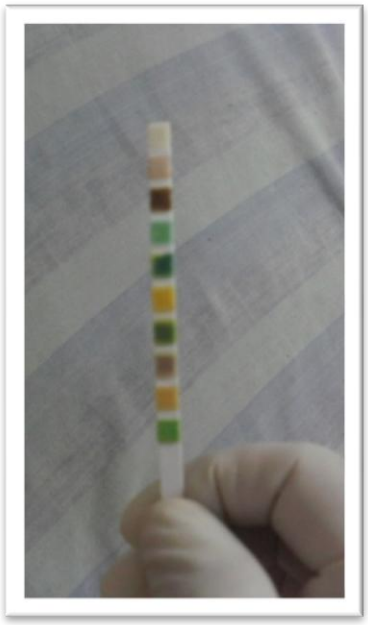
Strip test shows the how esterase catalyses the hydrolysis of indolecarboxylic acid. The indoxyl combines to diazonium to form a violet azole.[21]The esterase requires 5 minutes.

As per the criterias for ascitic fluid testing, strip introduced in to the fluid, followed by 120 seconds the change in the colour was matched with the chart in the container.

The stick is noted as either –ve or +ve.

SBP diagnosed if dipstick turns blue or violet in colour.

LERS STRIP – NO COLOUR CHANGE LERS



STRIP – COLOUR CHANGE TO PURPLE

Strip test color light blue/RED [grade 2: >125 polymorphonuclear leucocytes (PMNL)/ μL] or it turned purple/VIOLET (grade 3: >500 PMNL/μL).

MACROSCOPIC APPEARANCE

Colour and appearance of the fluid was noted-whether *crystal*
clear, transparent or slightly yellow, cloudy yellow, bloody, opaque and chylous, dark brown.

TRANSPORTNG THE SAMPLES

15 ml of fluid each was inoculated into 40 ml of Brain Heart

Infusion (BHI) broth and 40 ml of Thioglycollate broth at the bedside respectively. The BHI and the thioglycollate broth bottles were immediately transported to the laboratory and incubated at 36°c.Another 4ml was collected in a sterile screw capped test tube and sent to the microbiology laboratory .

PROCESSING SAMPLES

When the test tube was received in the microbiology laboratory, conventionally, centrifugation speed was 3000 revolutions/10 minutes and centrifuged sample was inoculated into BHI broth in the laboratory , followed up and processed along with the BHI broths inoculated at the bedside[15].

MICROSCOPIC EXAMINATION

From the sediment direct gram stain, acid fast stain was performed.

BACTERIAL CULTURE

A part of the sediment was lysed *with Triton X*-at room temperature, kept 5 minutes and inoculated into Blood agar, Mac Conkey agar incubated aerobically at 37 °c for 48 hrs, Chocolate agar which incubated at 37 °c in 5% CO2 for 48 hrs, Brain heart infusion agar was incubated for 48 hrs.

The broth bottles were followedfor 7 days with aerobic and anaerobic subculturing at 24, 48 and 168 hrs. Additionally, subculture from bedside BHI broth onto 2% Tween 80 BAP was done[11].

CONVENTIONAL BHI VS BEDSIDE BHI

IDENTIFYING ISOLATES

The preliminary tests-motility, catalase, oxidase Gramstain, were performed on all isolates and based on the results further identification of the isolates were done by standard microbiological tests.

ANTIMICROBIAL SUSCEPTIBILITY

The Isolatessubjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method. The panel of antibiotics that were used with zone diameters used for interpretation is given in annexure (Appendix).

Mueller-Hinton agar (MHA) plate is inoculated with 0.5 McFarland standard of the isolate to get a lawn culture. Using sterile forceps, the antibiotic discs were placed over the agar surface, incubated at 37°C in ambient air for 16 to 18 hrs. The results were interpreted as per Clinical Laboratory Standards Institute Guidelines (CLSI Guidelines 2012).

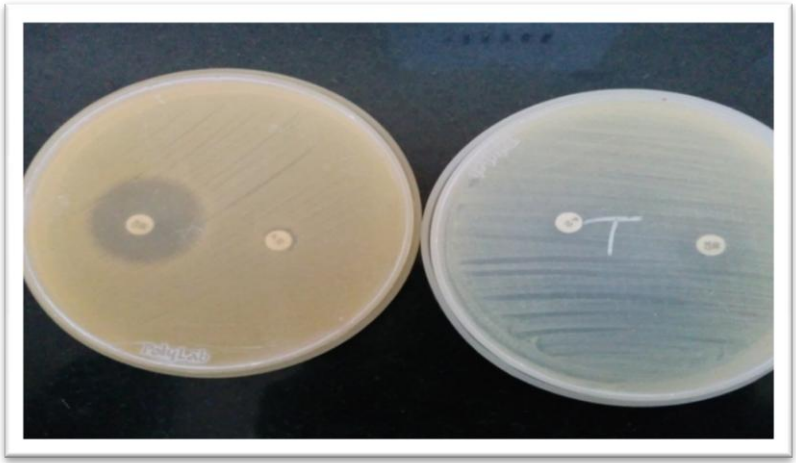
The Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 were used as quality control strains. The isolates were also tested for Methicillin resistance by Cefoxitin disc diffusion method, for ESBL production by Phenotypic Confirmatory Test and Carbapenamase production by Modified Hodge Test. (CLSI Guidelines 2012).

YELLOW PIGMENTED PSEUDOMONAS

CEFOXITIN DISC DIFFUSION METHOD

0.5 McFarland standard of the test isolate inoculated as lawn culture onto Mueller Hinton agar. Cefoxitin disc *(30 µg)* was applied and incubated overnight at 35oC for 16-18 hrs. Zone size 21 mm indicates MRSA and Zone size of 24 mm indicates MRS.

CEFOXITIN DISC DIFFUSION METHOD SHOWING MRSA



PHENOTYPIC CONFIRMATORY TEST

0.5 McFarland standard of the test isolate inoculated as lawn culture onto Mueller Hinton agar. Tested by combined disc diffusion method using *Ceftazidime (30µg) and Ceftazidime-clavulanic acid (30/10µg)* and incubated overnight at 37°C for 16-18 hrs. ESBL production was seen when there is 5 mm in zone diameter for the combined disc than when ceftazidime disc was tested alone.

MODIFIED HODGE TEST

1:10 diluted 0.5 McFarland standard of ATCC Escherichia coli (25922) inoculated as lawn culture onto Mueller Hinton agar.*10µg of Ertapenem/Meropenem disc* was placed in the centre.3-5 colonies of the test isolate was inoculated in straight line out from the edge of the disc.The plate was incubated overnight at 37oC for 16-20 hrs. If there was, enhanced growth of ATCC Escherichia coli around the streak line of test isolate, the isolate was positive for carbapenemase production.

CARBAPENAMASE PRODUCTION – MODIFIED HODGE TEST

FUNGAL AND MYCOBACTERIAL CULTURE

Sabouraud's dextrose agar was inoculated and incubated at 25°C for 10 days and examined daily for growth , Lowenstein Jensen Medium was inoculated and incubated at 37°C for 8 weeks examined twice weekly for growth respectively (in clinically relavent cases)11.

MOLECULAR METHOD

About 2ml of ascitic fluid was aliquoted and stored at -70°C for PCR – amplification of 16S rRNA gene to test for the presence of bacterial DNA in Culture Negative Neutrocytic Ascites (CNNA).Randomly selected Culture Negative Non-Neutrocytic Ascites (CNNNA) were used as controls.

ASCITIC FLUID - CELL COUNT

About 4 ml of ascitic fluid was placed in a tube containing the anticoagulant EDTA (ethylenediaminetetraacetic acid) for cell count (both total and differential). To another 5ml of freshly tapped ascitic fluid,

Leucocyte Esterase Reagent Strip (LERS) / NITRITE reagent strip test was dipped and immediately removed and its colour compared with the colour chart.

PMN COUNT/µl COLOUR SCALE

GRADE	PMNCOUNT/µl	COLOUR SCALE
0	>15	NoChange(NC)
1	>70	Lightyellow(Y)
2	>125	Lightblue(B)
3	>500	Purple(P)

The results obtained from using the LERS/nitrite reagent strip test was compared with the PMN count obtained from the laboratory.

ASCITIC FLUID-BIOCHEMISTRY

About 5 ml of ascitic fluid was placed in a tube and sent for biochemistry to estimate Glucose, Total protein, albumin (routine), amylase and lactate dehyrogenase (in clinically relevant cases).

FOLLOW UP

Repeat paracentesis was done after 48 hours in culture positive and CNNA patients to check for 30% decrease in PMN Count from the baseline which signifies resolving infection.

STEPS FOR DNA EXTRACTION

DNA extraction was done using Purefast bacterial Genomic DNA minispin prep kit.

1. 200 µl of sample was added to 1.5 ml centrifugetube and centrifuge at 12000 rpm for 5 min.

2. The supernatant was discarded and 400 µl of lysis buffer was added to the pellet in the centrifuge tube and mixed well.

3. 20 µl of Proteinase K was added to the centrifuge tube and mixed well.

4. The centrifuge tubes were incubated at 56°c for 15 min.

5. 200 µl of Isopropanol was instilled and kept in Normal temperature for the duration of five minutes.

6. Entire sample INTRODUCED to purefast and centrifuged 12,000 rpm for one min.

7. The flow of the sample residue was removed and spin sample keptinto the collection tube.

8. 500 µl of Wash Buffer-I was added to the purefast spin column and centrifuged at 12,000 rpm for 1 min.

9. The flow-through was discarded and the spin column was placed into the same collection tube.

10. 500 µl of Wash Buffer-II was added to the purefast spin column and centrifuged at 12,000 rpm for 1 min.

11. The flow-through was discarded and the spin column was placed into the same collection tube.

12. Empty spin - the empty spin column with collection tube was centrifuged for additional 1 min.

13. The spin column was transferred into a fresh 1.5 ml centrifuge tube.

14. 50 µl of pre-warmed Elution buffer (56°c) was added to the centre of the spin column and incubated for 2 min at room temperature.

15. The spin column was centrifuged at 12,000 rpm for 1 min and the column was then discarded.

16. Eluted DNA was present in the centrifuge tube and stored at -20 °c for later analysis.

POLYMERASE CHAIN REACTION

Before use all the reagents were thawed completely, mixed and centrifuged briefly.

MASTER MIX

dNTP mix 10 mM - 0.5 µl

10x Taq buffer - 3 µl

Taq polymerase enzyme - 0.4 µl

10x red dye - 2 µl

Nuclease free water - 14.1 µl

Total - 20 µl

DETECTION MIX

COMPONENTS	TEST SAMPLE
PCR Mastermix	20µl
Universal bacterial 16Sr RNA primermix	5µl
Purified DNA sample	5µl
Total reaction volume	30µl

Negative control:

Five µl of water is added in the place of purified DNA.

Positive control

5 µl of positive control (ATCC Escherichia coli 25922) was added in the place of purified DNA.

The PCR vials are centrifuged briefly before placing into thermal cycler.

AMPLIFICATION PROTOCOL

STEPS	TIME	TEMPERATURE	CYCLES
Initialdenaturation	4min	95°C	1cycle
Denaturation	30sec	95°C	35cycles
Annealing	30sec	55°C	
Extension	30sec	72°C	
Finalextension	5min	72°C	1cycle

ANALYSIS BY GEL ELECTROPHORESIS

Preparation of agarose gel

2% agarose gel was prepared by adding 2 gms agarose powder to 100ml of electrophoresis buffer, then heated in a microwave oven for 2 min, mixed well until the agarose was uniformly dissolved.After coolingto about 60°C 5 µl ethidium bromide was added to 100 ml of the gel tofacilitate visualization of DNA after electrophoresis. Ethidium bromide being a mutagen was handled with caution.

A well-former template (COMB) was placed across the end of the casting tray and the solution was then poured into the casting tray which acted as a mold and allowed to solidify.

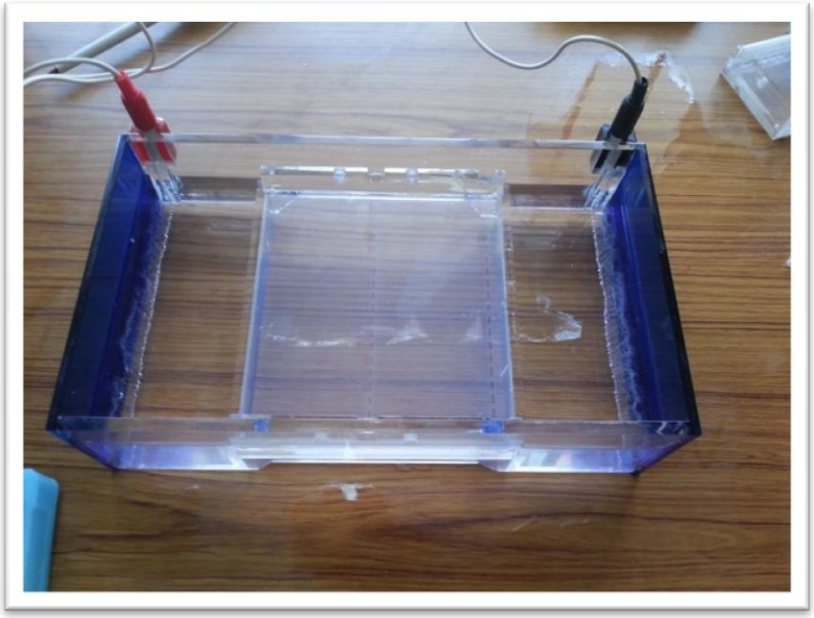
Electrophoresis (TAE-Tris Acetic acid-EDTA) buffer was poured into the electrophoresis tank.After the gel hardened enough, the gel was mounted on the electrophoresis tank so that the gel was completely immersed. The comb was then carefully removed.

Gel electrophoresis 10 µl of each sample, negative and positive controls were loadedinto the respective wells using a micropipette. 10 µl of 100 base pairDNA ladder was also loaded into the respective well using amicropipette.

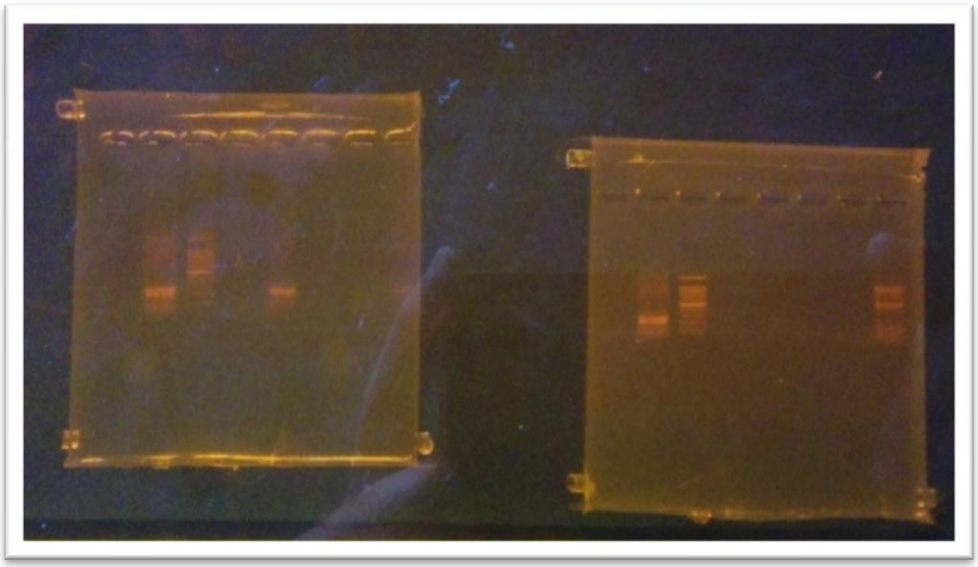
Electrical leads were connected. The buffer served as conductor of electricity and also controlled the pH. Constant voltage of 50-100 V was applied for the gel to run until the ethidium bromide dye had migrated ¾ the length of the gel. The negatively charged DNA if amplified by PCR migrated from cathode to anode.

The PCR products after the gel electrophoresis were observed under UV transilluminator. Amplicons of size 250-500 bp were consistent with bacterial DNA amplification.

PCR - AGAROSE GEL ELECTROPHORESIS



UV TRANSILLUMINATOR SHOWING 16S rRNA GENE AMPLIFICATION



OBSERVATION AND RESULTS

Ascitic fluid was tapped from 422 Decompensated Liver Disease (DCLD) patients (141 Outpatients and 281 Inpatients) under aseptic precautions. Acid fast stain ,Direct gram stain, were performed in the centrifuged deposit of the samples.

Bacterial and fungal culture is done for the ascitic fluid samples and the isolates are identified by standard microbiological tests. Antimicrobial susceptibility testing is done for the significant isolates according to CLSI Guidelines 2012.

Culture for Tuberculosis is done only in clinically relevant cases. PCR for the detection of bacterial DNA is performed in the ascitic fluid of 20 CNNA (Culture Negative Neutrocytic Ascites) samples and 20 selected randomly CNNNA (Culture Negative Non-Neutrocytic Ascites) samples.

Univariate analysis of the data was done by Pearson Chi-Square Test and Fisher's Exact Test. The p values less than 0.01 were considered as highly stastiscally significant ($p < 0.01$).The p values less than 0.05 were considered as stastistically significant ($p < 0.05$) and Study results are presented as follows:

TABLE 1: DISTRIBUTION OF AGE IN DCLD PATIENTS (n=422).

Age in Years	Outpatients (n=141)				Inpatients (n=281)			
	Male		Female		Male		Female	
	n=117	%	n=24	%	n=253	%	n=28	%
11-20	-	0	2	1.4	4	1.4	4	1.4
21-30	10	7.1	3	0	18	6.4	2	0.7
31-40	14	10	3	2.9	37	12.9	2	0.7
41-50	41	28.8	4	4.3	113	40	6	2.1
51-60	51	35.7	12	8.6	57	20	12	4.3
61-70	1	0	-	0	27	9.3	2	0.7
71-80	2	1.4	-	0	-	0	-	0
TOTAL	117	83	24	17	253	90	28	10
	141				281			
	422							

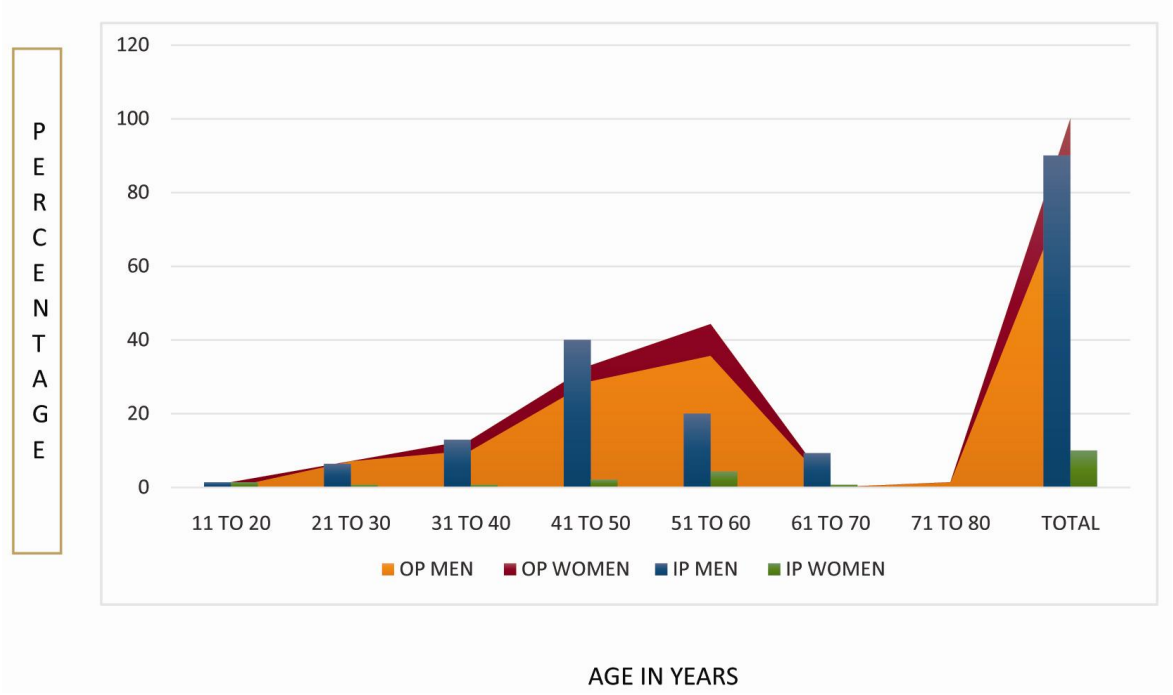


TABLE 1:DISTRIBUTION OF AGE IN DCLD PATIENTS (n=422)

Most of the DCLD patients are in the age group of 50-61 yrs, then followed by the age group of 40-51 yrs.The mean age was 46.33 yrs and the median age was 47yrs. Many of them are males.

TABLE 2:ETIOLOGY AND SEX DISTRIBUTIONPATTERN OF DCLD PATIENTS:(n=422)

ETIOLOGY	Out patients (141)				Inpatients (281)			
	MALE		FEMALE		MALE		FEMALE	
	n=117	%	n=24	%	n=253	%	n=28	%
Alcohol	96	65.7	-	0	155	55.1	1	0.3
HBV	6	4.2	2	1.4	31	11.0	1	0.3
HCV	2	1.4	2	1.4	12	4.2	1	0.3
Cryptogenic	4	2.8	20	14.1	8	2.8	19	6.7
Alcohol+HBV	4	2.9	-	0	16	5.6	-	-
Alcohol+HCV	1	0.7	-	-	9	3.2	-	-
Alcohol+HBV+HCV	1	0.7	-	-	2	0.7	-	-
Alcohol+Other Causes	3	2.1	-	0	18	12.7	-	-
HBV+ Other causes	-	-		-	2		-	-
TOTAL	117		24		253		14	

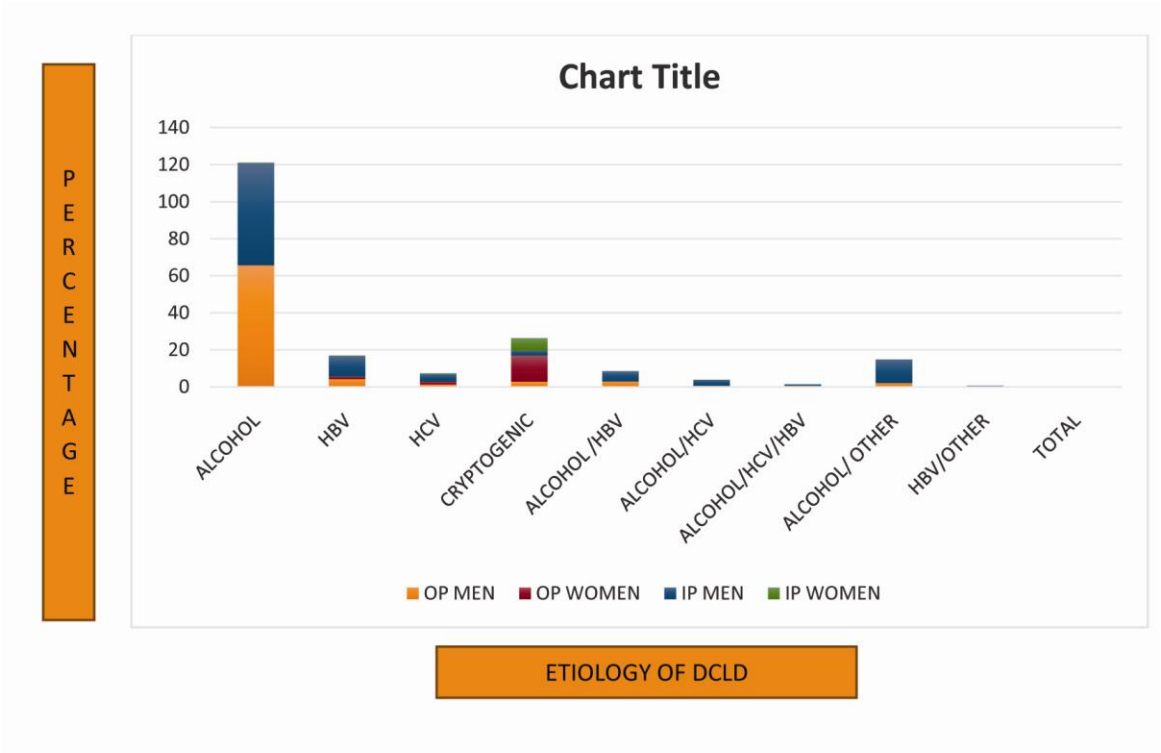


TABLE 2

ETIOLOGY AND DISTRIBUTION OF SEX PATTERNS OF DCLD PATIENTS:(N=422).

Alcoholic LiverDisease leading to cirrhosis is the frequent cause of ascites among men in outpatient (66.7%) and inpatient (54.1%)category whereas,Cryptogenic cirrhosis is frequent cause among women in both the categories with 13.1% in outpatient and 8.7% in in patient.

TABLE 3: SIGNIFICANT CLINICAL FEATURES IN CIRRHOTIC PATIENTS AND IN PATIENTS PRESENTING WITH AFIS: (N =158)

SIGNS & SYMPTOMS	Outpatients		Inpatients	
	(141)	AFIs (n=34)	281	AFIs (n=117)
Abdominal Pain(AP)	4(3%)	2(5.8%)	14(5%)	4(3.3%)
UGI Bleeding (UGB)	-	-	50(19.2%)	35(24%)
Diarrhoea (D)	-	-	21(7.1%)	4(3.3%)
Hepatic Encephalopathy (HE)	-	-	26(9.3%)	10(8%)
Fever (F)	6(4.2%)	6(17.6%)	48(17.1%)	34(27.4%)
Asymptomatic(A)	131(93%)	26(76.6%)	78(28%)	6(4.8%)
AP+UGB+F	-	-	2(0.7%)	2(1.6%)
AP+F	-	-	8(3%)	4(3.3%)
UGB+F	-	-	4(1.4%)	4(3.3%)
UGB+HE	-	-	6(2.1%)	6(4.8%)
UGB+HE+F	-	-	4(1.4%)	4(3.3%)
HE+F	-	-	16(5.7%)	16(12.9%)
TOTAL	141	34	140	117
	422			

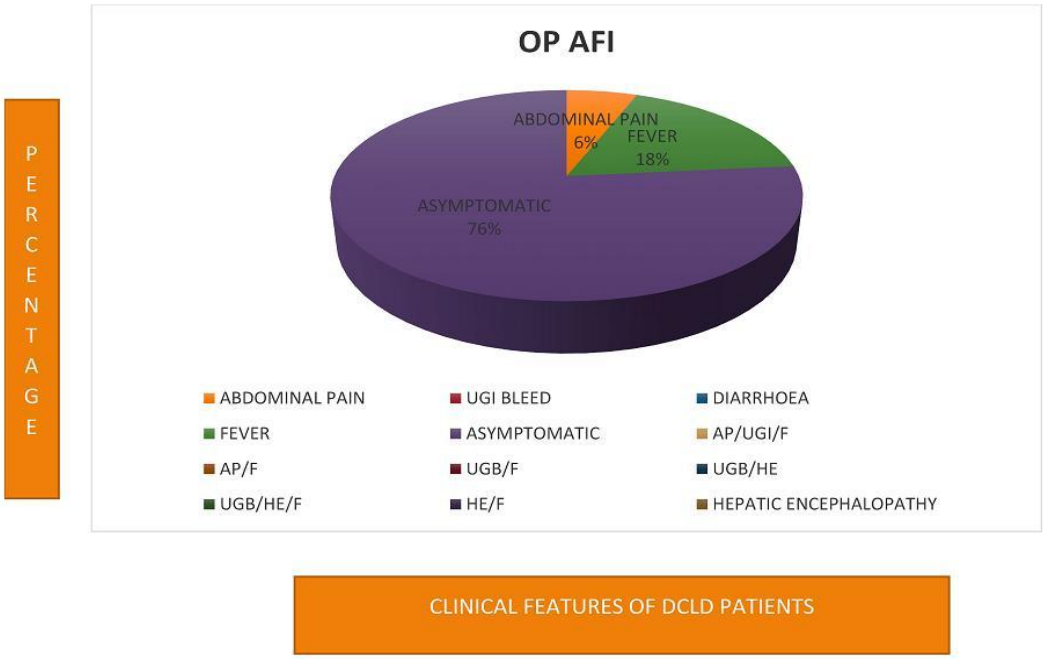


TABLE 3

SALIENT CLINICAL FEATURES OF DCLD PATIENTS AND IN THOSE PRESENTING WITH ASCITIC FLUID INFECTION: (N =158)

AFIs-Ascitic Fluid Infections.

Most of the outpatients are asymptomatic (94%). Among the symptomatic inpatients Upper Gastrointestinal bleeding, is the major cause.Fever was the major symptom among inpatients with AFIs.(25.4%).

TABLE 4:LABORATORY PARAMETERS INCIRRHOTIC PATIENTS: (n=422)

Laboratory Parameters		Outpatients (n=141)	Inpatients (n=281)	Total (n=422)
Leukocyte count	>11,000/mm^3	2	48	50(12%)
	≤11,000/mm^3	138	232	370(88%)
Platelet count	<98,000/mm^3	10	66	76(18%)
	≥98,000/mm^3	130	214	344(82%)
Serum bilirubin	<2.5 mg/dl	108	106	214(51%)
	≥2.5 mg/dl	32	174	206(49%)
Serum albumin	≤ 3.5 g/dl	116	230	346(82.4%)
	>3.5 g/dl	24	50	74(17.6%)
Serum Creatinine	≥2.5 mg/dl	2	8	10(2.4%)
	1.5-2.4 mg/dl	2	16	18(4.3%)
	<1.5 mg/dl	136	256	392(93.3%)
BUN BUN	LESS THAN 25	26	79	105(24.8%)
	MORE THAN 25	116	203	319(75.5%)
	LESS THAN 130	35	125	260(61.6%)

SODIUM	MORE THAN 130	107	157	264(62.5%)
POTASSIUM	LESS THAN 4.5	121	205	326(77.2%)
	MORE THAN 4.5	21	76	97(22.9%)
SAAG ratio	≤1.1 g/dl	23	41	64(15.1%)
	>1.1 g/dl	121	240	361(85.5%)

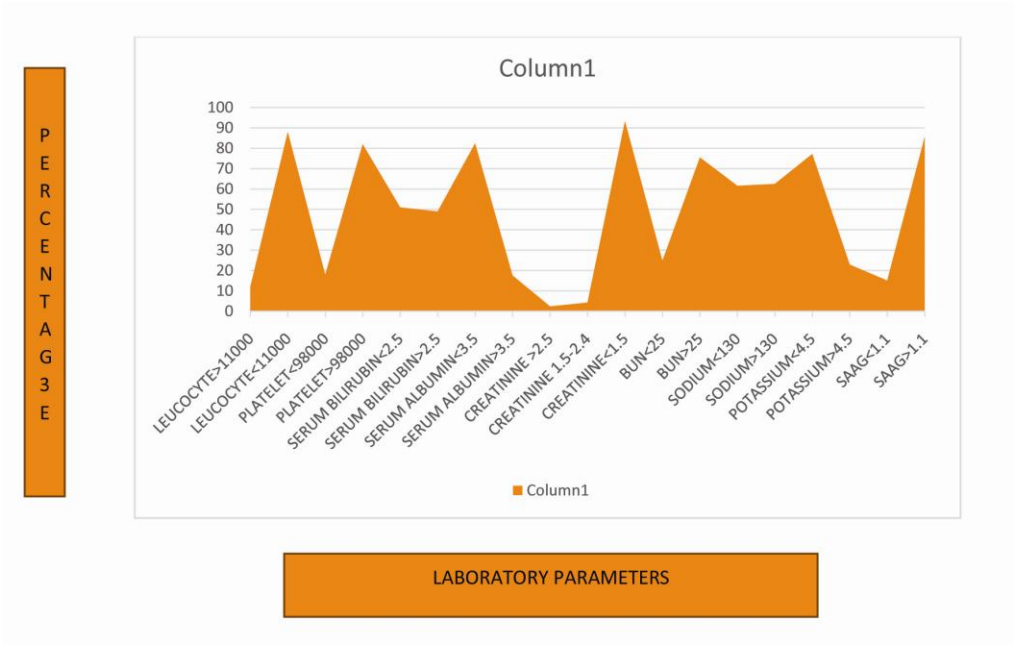
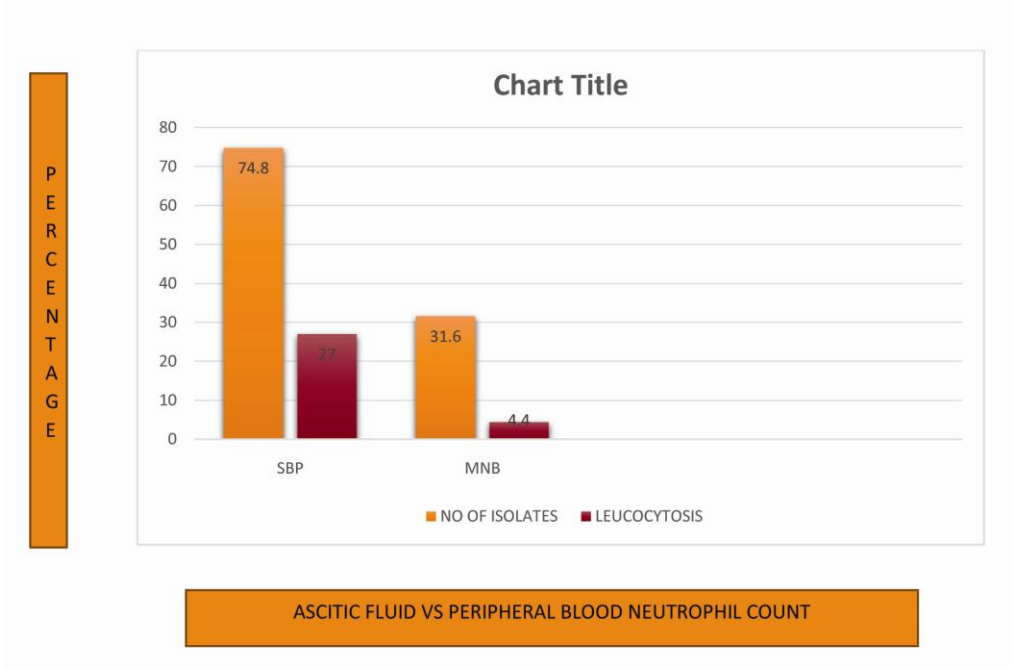


TABLE 4 LABORATORY PARAMETERS AMONG DCLD PATIENTS:

Leukocytosis was seen in only 13% of the total patient population. Creatinine levels (>1.5 mg/dl) suggestive of HRS (Hepato Renal Syndrome) was seen in 28 patients. Majority of our patients had (83.2%) High SAAG ascites.

TABLE 5: COMPARISON OF BLOOD NEUTROPHIL COUNTVSASCITIC FLUID IN PATIENTS WITH AFIS:

Ascitic fluid PMN count	AFIs	No. of isolates	Leukocytosis (>11,000) with Neutrophils >60%
≥250/mm^3	SBP	113(74.8%)	27
<250/mm^3	MNB	51(31.6%)	3

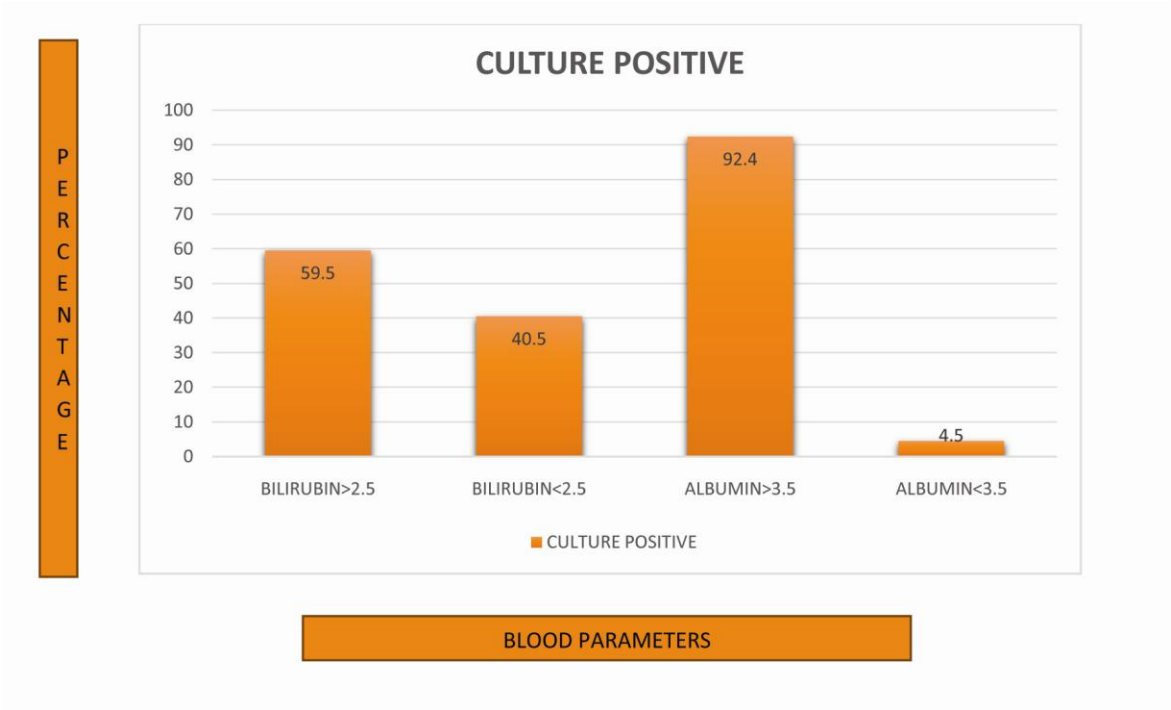


ASCITIC FLUID VS PERIPHERAL BLOOD NEUTROPHIL COUNT

The ascitic fluid neutrophil count was elevated (>250/mm3) in 113 (69.4%) of AFIs among which only 22 had elevated peripheral blood neutrophil count. There was no correlation between both and this observation was highly statistically significant.

TABLE 6: CULTURE POSITIVES IN CIRRHOTIC PATIENTS AND RISK FACTORS FOR DEVELOPING ASCITIC FLUID INFECTIONS (AFIS) (n=158)

SERUM INDICES		CULTURE NEGATIVES	CULTURE POSITIVES	SIGNIFICANCE
Bilirubin level	≥ 2.5 mg/dl (206)	112	94(59.5%)	P value-0.0227
	< 2.5 mg/dl (214)	150	64(40.5%)	
Albumin	≤ 3.5 g/dl (372)	226	146(92.4%)	P value-0.2624
	> 3.5 g/dl (48)	36	12 (7.6%)	

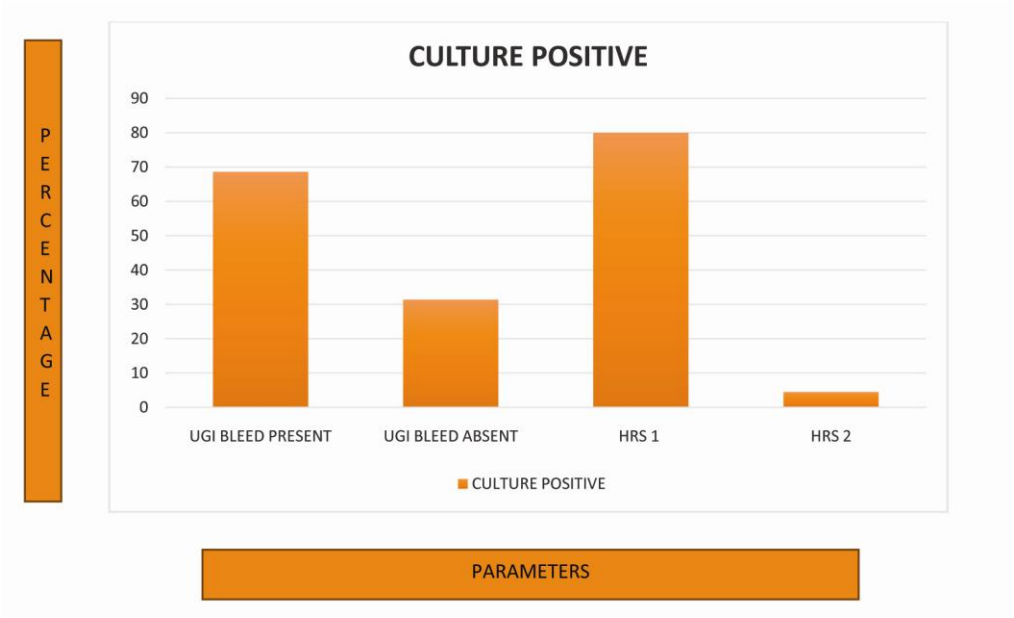


The prevalence of Ascitic Fluid Infections (AFIs) in DCLD

Patients having raised serum bilirubin (>2.5 mg/dl) and lowered albumin<3.5 g) were 58.5%and 91.4%.

TABLE 7: CULTURE POSITIVES AMONG CIRRHOTICS PATIENTS WITH UGI BLEEDING / HEPATO RENALSYNDROME (n=422)

CLINICAL STATUS		TOTAL (n=422)	CULTURE POSITIVES	SIGNIFICANCE
H/O UGI Bleeding	Present	70	48 (68.6%)	p = 0.0001
	Absent	350	110(31.4%)	
HRS-I	Creatinine ≥2.5 mg/dl	10	8(80%)	p = 0.0097
HRS-II	Creatinine 1.5-2.4 mg/dl	18	12(66.9%)	

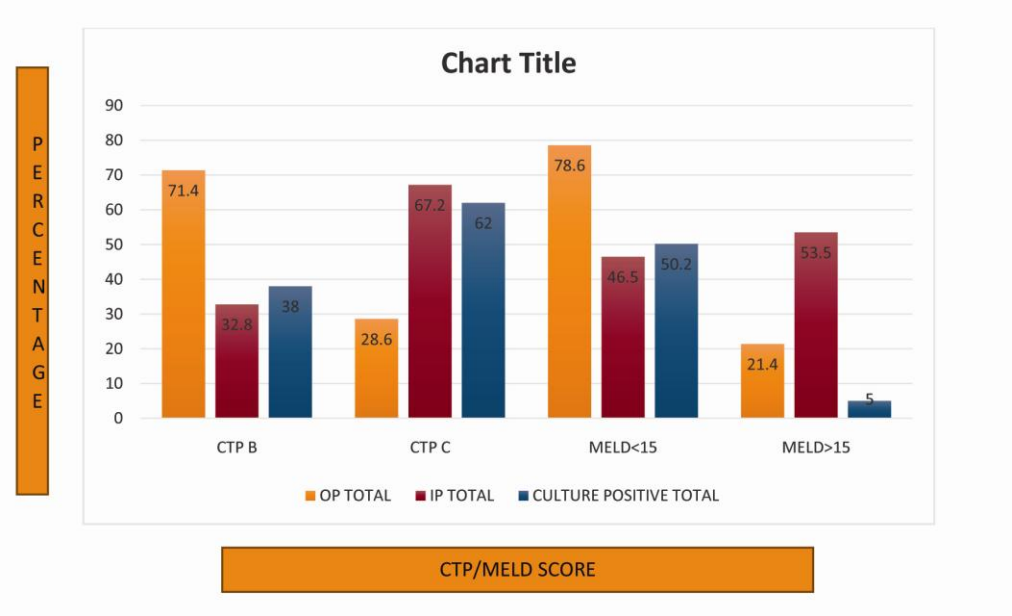


The prevalence of AFIs in DCLD patients with UGIbleeding, HRS-I and HRS II were 67.6%, 80.1% and 66.8%.

TABLE 8:MELD AND CTP SCORE AMONG DCLD PATIENTS WITH ASCITIC FLUID INFECTIONS (n=158)

Score		Total		Culture positives (n=151)		
		Op (n=141)	Ip (n=281)	Op (n=34)	Ip (n=124)	Total
CTP Score	(7-9) Class B	100 (71.4%)	92 (32.8%)	24	36	60 (38%)
	(10-15) Class C	40 (28.6%)	188 (67.2%)	10	88	98 (62%)
MELD Score	Low(<15)	110 (78.6%)	130 (46.5%)	26	54	80 (50.2%)
	High(≥15)	30 (21.4%)	150 (53.5%)	8	70	78 (49.8%)

Ip-Inpatients,Op-Outpatients.



CTP*-CHILD TURCOTTE PUGH SCORE: Most of the outpatients belonged to Class B(72.4%) while most of the inpatients belonged to Class C (68.2%). The prevalence of AFIs in DCLD patients belonging to CTP Class B was 37% and Class C was 60%. (p=0.0878)

MELD* - MODEL FOR END- STAGE LIVER DISEASE (MELD) SCORE: Majority of the outpatients had low score and inpatients 52.5% is the high score (>15) and 45.5% had low score (<15).The AFIs is of equal prevalence in DCLD patients with high (49.7%) and low (50.3%) MELD scores. (p=0.1518)

TABLE 9: ASCITIC FLUID INFECTIONS (AFIS) IN CIRRHOTICS OF VARIED ETIOLOGIES.

SAAG	ETIOLOGICAL FACTORS	CULTURE POSITIVES		
		GPC	GNB	Candida
Low ≤1.1 g/dl (62)	Alcohol	6	4	2
	Alcohol/HBV	-	2	-
	Alcohol/Malignancy	-	2	-
	Post Whipple's (Hypoalbuminemia)	-	2	-
High >1.1 g/dl (358)	Alcohol	30	60	-
	Alcohol/HBV	-	8	-
	Alcohol/HCV	-	2	2
	Alcohol/HBV/HCV	-	2	-
	Cryptogenic	12	8	-
	Budd-Chiari Syndrome	-	4	-
	Cholestatic jaundice	-	4	-
	Wilson's Disease	-	2	-
	Non Alcoholic Fatty Liver Disease	-	2	-
	Non Cirrhotic Portal Fibrosis	2	-	-
	HBV/SOL Rt.lobe of Liver(?HCC)	-	2	-
TOTAL		50	108	4

HCC-Hepatocellular Carcinoma SOL-Space Occupying Lesion.
This table reveals that AFIs are present in 38 cases of DCLD of other etiologies apart from cirrhosis.

TABLE 10: MICROBIAL PROFILE ISOLATED AMONG OUTPATIENTS AND INPATIENTS WITH DCLD

PATIENT CATEGORY		CULTURE POSITIVES	GPC	GNB	Candida	M.TB
OUTPATIENTS (141)	Males(116)	24	18	6	-	-
	Females(24)	10	6	4	-	-
TOTAL		34(24%)	24(75%)	10(25%)		
INPATIENTS (281)	Males(252)	112	22	86	4	2
	Females(48)	12	4	8	-	-
TOTAL		124(44.3%)	26(21%)	94(75.8%)	4(3.2%)	2
SIGNIFICANCE		p = 0.005	p = 0.0003			

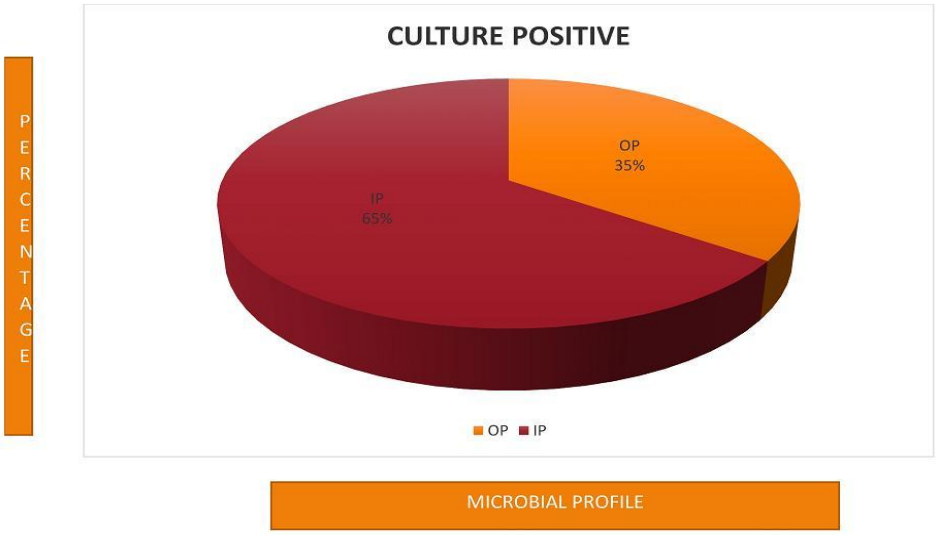


TABLE 10:MICROBIAL PROFILE ISOLATED AMONG OUTPATIENTS AND INPATIENTS WITH DCLD:

AFIs are more prevalent among inpatients (43.3%) than outpatients (23%) (p=0.005). In DCLD outpatients, majority (74%) of AFIs were mainly due to Gram positive organisms, whereas in DCLD inpatients majority (74.8%) of AFIs were due to gram negatives. (p = 0.0003) In addition to 158 culture positives in AFIs ,there 2 cases of Tuberculous Peritonitis. The Tubercle bacilli was absent in AFB Smear but LJ medium culture yielded growth in 5 weeks. Faster results with sensitivity was obtained by MGIT.

MGIT-Mycobacterial Growth Indicator Tube.

TABLE 11: CLASSIFICATION OF ASCITIC FLUID INFECTIONS IN CIRRHOTICS PATIENTS VARIANTS OF AFIs

VARIANTS	TOTAL	Culture Positives		
		GPC	GNB	Candida
Spontaneous bacterial peritonitis (SBP)	108 (68.4%)	20	86	2
Monomicrobial non-neutrocytic bacterascites (MNB)	50 (31.6%)	30	18	2
TOTAL	158(100%)	50(31.7%)	104 (65.8%)	4(2.5%)
Culture Negative Neutrocytic Ascites (CNNA)	20	-	-	-

Among the 158 Culture Positive AFIs in DCLD patients based on PMN count 108 (68.4%) were categorized as SBP and 50 (31.6%) were
categorized as MNB. Among the isolates GPC was found in 31.7%, GNB
in 65.8% and Candida in 2.5% of AFIs respectively .GPC was predominant among the MNB category and GNB was predominant among the SBP category. (p = 0.0004)

TABLE 12: COMPARING DIRECT GRAM STAIN FINDINGS WITH CULTURE POSITIVES (n=158)

AFIs		DIRECT GRAM STAIN -PMNs				ORGANISM		Culture Positives
		Nil	Few (<10/HPF)	Moderate (11-40/HPF)	Plenty (>40/HPF)	+	-	
SBP 20	MNB 30	30	10	6	4	4	46	GPC (50)
SBP 86	MNB 18	18	16	56	14	18	86	GNB (104)
SBP 2	MNB 2	2	-	-	2	2	2	Candida (4)

HPF-High Power Field

PMNs were absent in Direct Gram Stain in all cases of MNB. In SBP plenty of PMNs were seen in candida infection, followed by GNB and GPC.

TABLE 13: COMPARING ASCITIC FLUID MACROSCOPY WITH DIAGNOSTIC YIELD-CONVENTIONAL VS BEDSIDE INOCULATION (n=422)

Macroscopy	No. Of samples (n=422)	Conventional-BHI broth	Triton-x	Bedside BHI broth	
				+BAP	+2% Tween80 BAP
Clear	378	10	36	112	126
Slightly turbid	10	4	4	8	8
Cloudy	14	12	12	12	14
Chylous	4	2	2	2	2
Muddy brown	6	4	4	4	4
Pale coloured	4	2	-	2	2
Haemorrhagic	4	2	2	2	2
Total	422	36 (8.6%)	60(14.3%)	142(33.8%)	158 (37.6%)
SIGNIFICANCE			p=0.0000	p=0.0000	p=0.0000

Cloudy, slightly turbid, chylous, muddy brown, pale and haemorrhagic ascitic fluids yielded culture growth even by conventional method, but clear ascitic fluid samples yielded more culture positives only when bedside inoculation technique was used. Among the 422 samples NO OBLIGATE ANAEROBES were isolated.

TABLE 14: MICROBIAL PROFILE ISOLATED BY EACH OF THE DIFFERENT CULTURE METHODS

Organism	Total n=151 (%)	Conventional-BHI broth	Triton-X	Bedside BHI broth	
				+BAP	+2% Tween 80 BAP
Klebsiella pneumoniae	40(25.3%)	6	26	36	40
Klebsiella oxytoca	8(5.1%)	8	8	8	8
Escherichia coli	20(12.7%)	8	10	20	20
Enterobacter aerogenes	10(6.3%)	2	2	8	10
Pseudomonas aeruginosa	8(5.1%)	2	2	6	8
Acinetobacter baumanii	4(2.5%)	-	-	-	4
Citrobacter freundii	2 (1.3%)	-	-	-	2
Citrobacter koseri	2(1.3%)	-	-	2	2
Pseudomonas luteola	4(2.5%)	-	-	-	4
Proteus species	4(2.5%)	2	2	4	4
Serratia marcescens	2(1.3%)	-	-	2	2
Staphylococcus aureus	20(12.7%)	2	4	20	20
CONS	14(8.8%)	-	2	14	14
Enterococcus faecalis	8(5.1%)	-	-	8	8
Enterococcus faecium	4(2.5%)	2	2	4	4
Streptococcus Group	1(1.3%)	2	-	2	1

A					
Streptococcus Group B	1(1.3%)	-	-	2	1
Candida albicans	2(2.5%)	2	2	4	2
TOTAL	158(100%)	36 (22.8%)	60 (38%)	140 (88.6%)	158 (100%)
SIGNIFICANCE			p=0.000	p=0.000	p=0.000

The organisms sequestered within the neutrophils and probably liberated by the surfactant action of 2% Tween 80 were Klebsiella pneumoniae(2) Enterobacter (1) Citrobacter freundii (1), Pseudomonas aeruginosa(1) and Pseudomonas luteola (2) and Acinetobacter baumanii(2).

TABLE 15:RESULTS OF LEUKOCYTE ESTERASE REAGENT STRIP(LERS) TEST COMPARED WITH GOLD STANDARD CELL COUNT IN AFIs

AF PMN count/mm^3	No.	Colour change				GPC (38)				GNB (116)				Candida (4)			
		NC	Yellow	Blue	Purple	N	Y	B	P	N	Y	B	P	N	Y	B	P
0-70	244	244 (100%)	-	-	-	10	-	-	-	6	-	-	-	-	-	-	-
71-125	28	20	8 (28.5%)	-		8	4	-	-	4	2	-	-	-	-	-	-
126-500	140	24	18	86 (61.4%)	12	6	4	2	4	14	4	62	4	4	-	-	-
>500	8	-	-	-	8 (100%)	-	-	-	-	-	-	-	8	-	-	-	-
TOTAL	420	288	26	86	20	38				116				4			

NC-No change, Y-Yellow, B-Blue, P-Purple

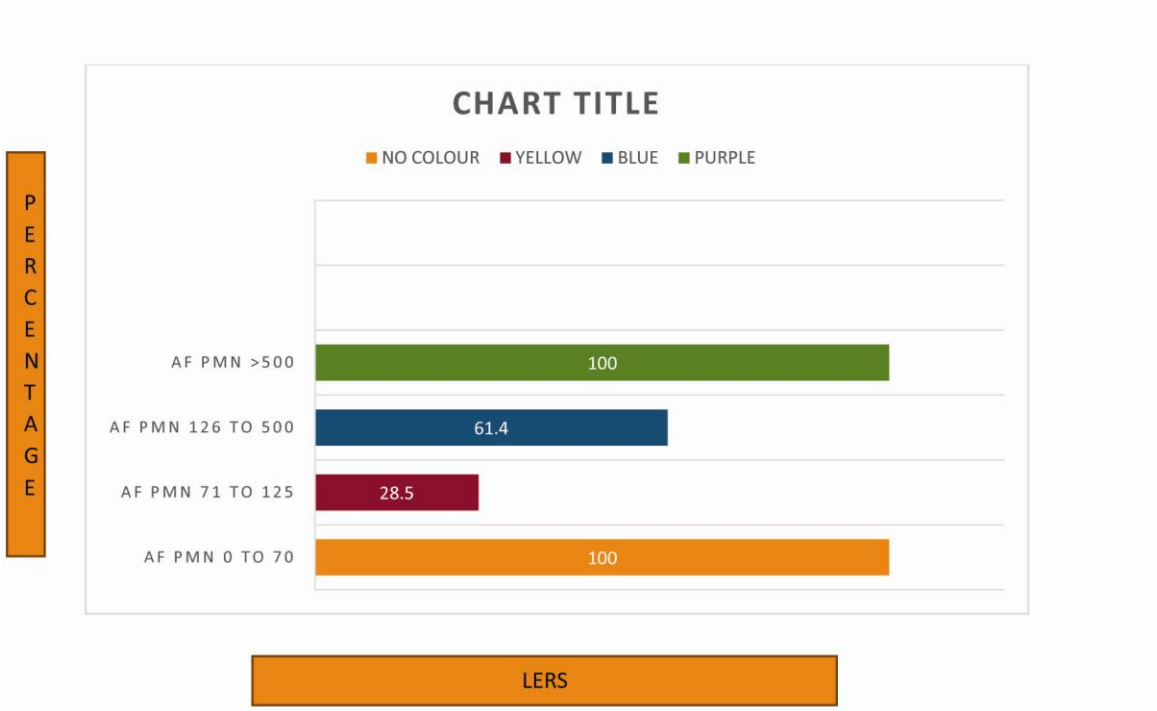


TABLE 15: RESULTS OF LEUKOCYTE ESTERASE REAGENT STRIP(LERS) TEST COMPARED WITH GOLD STANDARD CELL COUNT IN ASCITIC FLUID INFECTIONS:

TABLE 16 RESULTS OF LEUKOCYTE ESTERASE REAGENT STRIP(LERS) TEST COMPARED WITH GROWTH OF GRAM POSITIVE COCCI AND GRAM NEGATIVE BACILLI IN ASCITIC FLUID INFECTIONS:

AF PMN count/mm^3	No of patients	Blue colour change	Purple colour change	Blue GPC	Blue GNB	Purple GNB
126-500	140	86		2 (2.3%)	62 (72.09%)	
>500	8	-	8	-	-	8

The strip accurately identified PMN count in the lowest range (0-70) and the highest range (>500) with appropriate colour change, that is, with 100% accuracy .For the PMN counts in the ranges (71-125) and (126-500) the colour change was appropriate in 28.5% and 61.4% respectively.

TABLE 17:RESULTS OF NITRITE DIPSTICK COMPARED WITH GOLD STANDARD CELL COUNT IN AFIs:

AF PMN count/mm^3	No.	Colour change				GPC (38)				GNB (116)				Candida (4)			
		white	pink	red	violet	W	P	R	V	W	P	R	V	N	Y	B	P
0-70	244	244 (100%)	-	-	-	10	-	-	-	6	-	-	-	-	-	-	-
71-125	28	20	8 (28.5%)	-		8	4	-	-	4	2	-	-	-	-	-	-
126-500	140	24	18	86 61.4%	12	6	4	2	4	14	4	62	4	4	-	-	-
>500	8	-	-	-	8 (100%)	-	-	-	-	-	-	-	8	-	-	-	-
TOTAL	420	288	26	86	20	38				116				4			

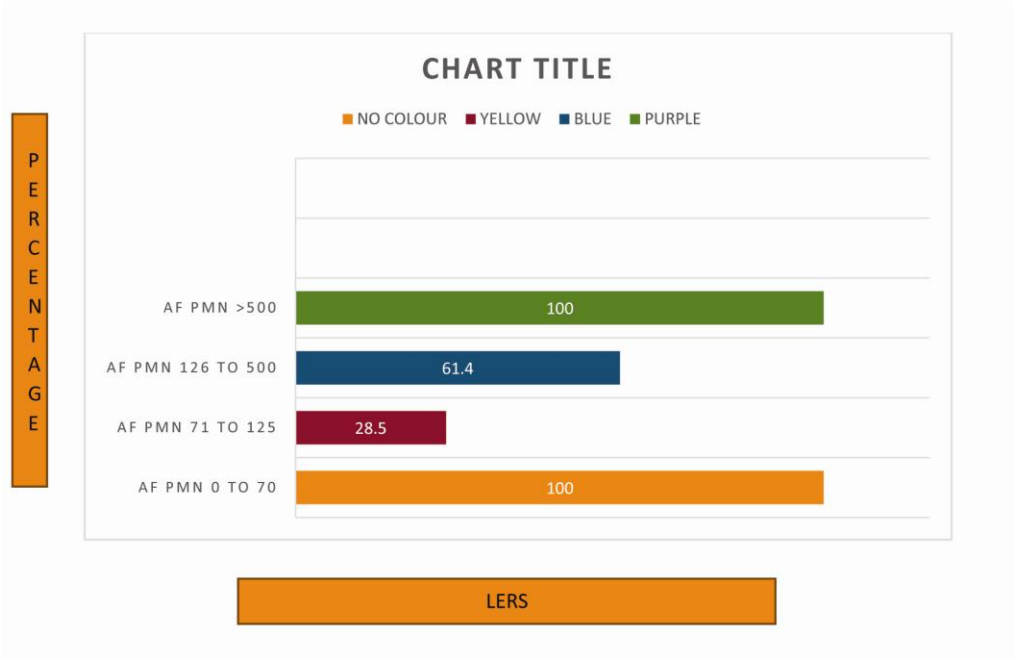


TABLE 17:RESULTS OF NITRITE REAGENT STRIP TEST COMPARED WITH GOLD STANDARD CELL COUNT IN AFIs:

TABLE 18 RESULTS OF NITRITE REAGENT REAGENT STRIP TEST COMPARED WITH GROWTH OF GRAM POSITIVE COCCI AND GRAM NEGATIVE BACILLI IN ASCITIC FLUID INFECTIONS:

AF PMN count/mm^3	No of patients	RED colour change	VIOLET colour change	RED GPC	RED GNB	VIOLET GNB
126-500	140	86		2 (2.3%)	62 (72.09%)	
>500	8	-	8	-	-	8

The strip accurately identified PMN count in the lowest range (0-70) and the highest range (>500) with appropriate colour change, that is, with 100% accuracy .For the PMN counts in the ranges (71-125) and (126-500) the colour change was appropriate in 28.5% and 61.4% respectively.

TABLE 19: ASCITIC FLUID ALBUMIN AND SUGAR LEVELS AMONG DCLD PATIENTS WITH ASCITIC FLUID INFECTIONS(n=158)

ASCITIC FLUID PARAMETERS		GPC	GNB	Candida	Culture Positives	SIGNIFICANCE
ALBUMIN	≤1.5 g/dl (272)	42	92	2	136(86%)	p=0.0001
	>1.5 g/dl (148)	8	12	2	22(14%)	
TOTAL PROTEIN	≤1.5 g/dl (180)	22	60	2	84(14%)	p=0.0217
	>1.5 g/dl (240)	28	44	2	74(47%)	
SUGAR	<50 mg% (106)	16	84	2	102(64.6%)	p=0.0002
	50-100 mg% (118)	24	14	0	38(24.1%)	
	>100 mg% (196)	10	6	2	18(11%)	

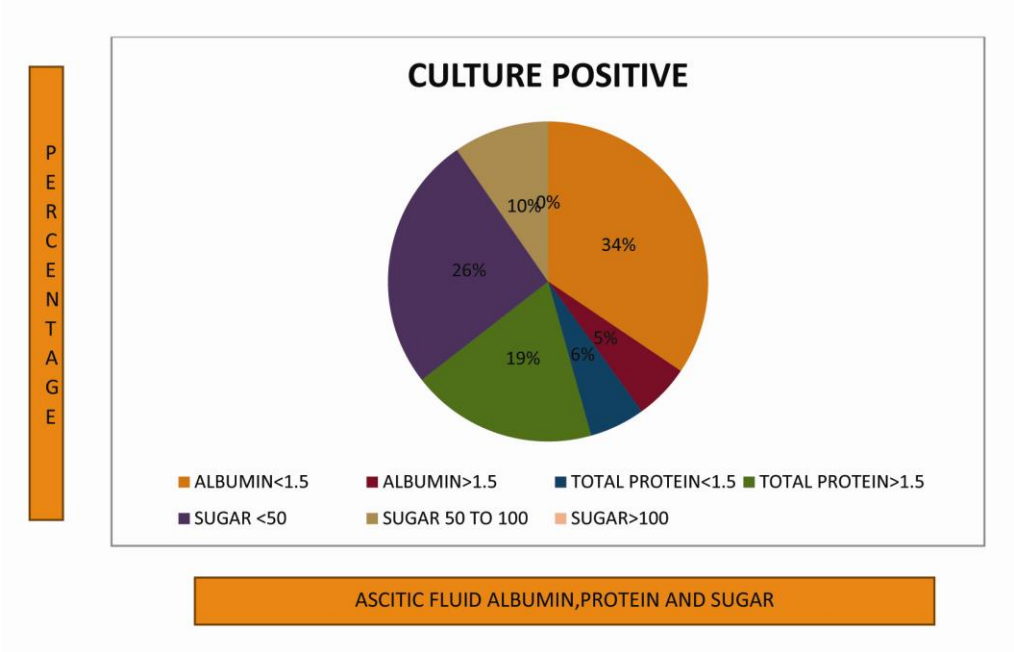


TABLE 19: ASCITIC FLUID ALBUMIN AND SUGAR LEVELS AMONG DCLD PATIENTS WITH ASCITIC FLUID INFECTIONS(n=158):

The prevalence due to AFIs among DCLD associated risk factors like decreased ascitic fluid albumin (<1.5g/dl) and total protein (<1.5g/dl) and low ascitic fluid sugar levels (<100 mg%) was found to be 82%, 55% and (64.7%+24.2%) 86.8% respectively.

TABLE 20: THE ANTIMICROBIAL SUSCEPTIBILITY PATTERN OFINPATIENTS WITH AFIS

ORGANISM	ANTIMICROBIAL AGENT														ESBL/ MBL
	Cefo or cefta (30µg)		Nor (10µg)		Cip (5µg)		Gen (10µg)		Ak (30µg)		Amc or PTZ (20/10µg) or (100/10µg)		Imi/ Mero (10µg)		
GNB	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
Kleb pneumoniae (17)	24	10	14	20	28	6	32	2	32	1	28	6	10	-	10
Kleb oxytoca (2)	0	4	0	4	0	4	2	2	4	0	4	-	4	-	4
Escherichia coli (10)	10	10	6	14	8	12	12	8	20	0	14	6	10	-	10
Enterobacter (5)	6	4	2	8	8	2	10	0	10	0	-		4	-	4
Pseudo aeruginosa(4)	4	4	2	6	6	2	8	0	8	0	8	0	4	-	4
Pseudo luteola(2)	2	2	2	2	4	0	4	0	4	0	4	0	2	-	2
Acinetobacter (2)	0	4	0	4	2	2	4	0	4	0	0	4	2	2	2/2

Citro freundii (1)	2	0	2	0	2	0	2	0	2	0	-		-	-	-
Citro koseri (1)	0	2	0	2	2	0	2	0	2	0	-		2	-	2
Proteus mirabilis (1)	2	0	2	0	2	0	2	0	2	0	2	0	-	-	-
Proteus penneri (1)	2	0	2	0	2	0	2	0	2	0	2	0	-	-	-
Serratia (1)	2	0	2	0	2	0	2	0	2	0	-		-		0
STAPH SPECIES (18)	Pen-G (10 U)		Cefox (30µg)		Nor (10µg)		Cip (5 µg)		Gen (10µg)		Ak (30 µg)		Doxy (30 µg)		MRS
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
Staph aureus(5)	4	6	2	8	2	8	8	2	8	2	10	0	6	4	8
CONS (4)	12	4	8	8	4	12	6	2	6	2	8	0	8	0	4
ENTEROCOCCUS (8)	Pen-G (10 U)		Doxy (30 µg)		HLG (120µg)		HLS (300µg)		Van (30 µg)		Linezolid (30 µg)		Quinupristin (15 µg)		VRE/ HLAR
Entero. Faecalis (4)	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
	4	4	8	0	8	0	4	0	4	0	-	-	-	-	-
Entero.faecium (4)	4	4	8	0	4	4	2	2	2	2	2	-	2	-	2/2

The prevalence of MRS, ESBL,VRE, HLAR and MBL among pathogens responsible for AFIs among inpatients were 66.7%, 41.4%, 27%,26% and 2.2% respectively.

TABLE 21: THE ANTIMICROBIAL SUSCEPTIBILITY OF OUTPATIENTS WITH AFIs

ORGANISM	ANTIMICROBIAL AGENT														ESBL/ MBL
	Cefo or cefta (30µg)		Nor (10µg)		Cip (5µg)		Gen (10µg)		Ak (30µg)		Amc or PTZ (20/10µg) or (100/10µg)		Imi/ Mero (10µg)		
GNB	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
Kleb pneumoniae (6)	6	0	6	0	6	0	6	0	6	0	6	0	-	-	-
Kleb oxytoca (4)	2	2	4	0	2	2	4	0	4	0	4	-	2	-	2
STAPH SPECIES(16)	Pen-G (10 U)		Cefox (30µg)		Nor (10µg)		Cip (5 µg)		Gen (10µg)		Ak (30 µg)		Doxy (30 µg)		MRS
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	2
Staph aureus (10)	8	2	8	2	6	4	8	2	8	2	10	0	10	0	
CONS (6)	6	0	6	0	2	4	4	2	4	2	6	0	6	0	-
ENTEROCOCCUS(4)	Pen-G (10 U)		Doxy (30 µg)		HLG (120µg)		HLS (300µg)		Van (30 µg)		Linezolid (30 µg)		Quinupristin (15 µg)		VRE/HLAR
Entero. faecalis (4)	S	R	S	R	S	R	S	R	S	R	S	R	S	R	-
	4	0	4	0	4	0	4	0	4	0	-	-	-	-	

The prevalence of MRS and ESBL among pathogens responsible for AFIs among outpatients were 12.6% and 21% respectively.

TABLE 22:MICROBIAL PROFILE OF DCLD INPATIENTS WITH HEPATIC ENCEPHALOPATHY(n=52)

HEPATIC ENCEPHALOPATHY (52)	CULTURE POSITIVES (36)	ORGANISMS (n=36)		
		UREASE POSITIVE (30)		UREASE NEGATIVE(6)
IP MALES (48)	32	26	Kleb-18	6
			Proteus-2	
			Pseudo-2	
			Acineto-2	
			Citro-2	
IP FEMALES (4)	4	4	Kleb-2	-
			Acineto-2	
SIGNIFICANCE		p=0.0078		

Among the 52 DCLD patients with Hepatic Encephalopathy, Ascitic fluid yielded culture positives in 37 samples and among them UREASE producing organisms were 31 (83.4%)

TABLE 23: COMPARING CLINICAL DATA AND LABORATORY DATA IN BACTEREMIC CIRRHOTICS INPATIENTS WITH AFIS (N=28)

CLINICAL DATA		GPC (n=4)	GNB (n=20)	CANDIDA (n=4)
AGE	30-40	-	4	2
	40-50	-	10	-
	50-60	2	6	2
	60-70	2	-	-
SEX	MALE	4	18	4
	FEMALE	-	2	-
GI BLEEDING	YES	4	14	2
	NO	-	6	2
PLATELET COUNT	<98,000	2	14	2
	≥98,000	2	6	2
PT	Elevated (>15 sec)	4	14	0
	Normal (≤15 sec)	-	6	4
INR	Normal (≤ 1.2)	-	6	2
	Elevated (>1.3)	4	14	2
LEUKOCYTOSIS (≥11,000)	PRESENT	-	8	0
	ABSENT	4	12	4

TOTAL BILIRUBIN	>2.5 mg/dl	2	8	4
	≤ 2.5 mg/dl	2	12	0
CREATININE	<1.5	4	12	4
	≥1.5	-	8	0
CTP	CLASS B	-	6	2
	CLASS C	4	14	2
MELD	<15	-	2	4
	≥15	4	18	0
OUTCOME	SURVIVAL	2	12	2
	DEATH	1	8	2

Among DCLD inpatients who had bacteremia along with AFIs,majority were males(92.8%),had history of gastrointestinal bleeding (71.3%),low platelet count (64.2%),elevated prothrombin time(64.1%) and INR (71.3%).In 50% of patients serum bilirubin was raised.Majority belonged to CTP Class C (71.3%) and had high MELD score (78.4%). About 12 (42.8%) patients with bacteremic AFIs succumbed to death.Gram negatives were isolated in 20 (71.5%),gram positives in 4 and candida in 4 (14.4%) cases respectively.

TABLE 24: COMPARISON OF PCR DETECTION OF 16S rRNA IN 20 CNNA AND 20 CNNNA SAMPLES:

AF 16S rRNA	CNNA (PMN Count ≥ 250/mm^3) (n=20)	CNNNA (PMN Count < 250/mm^3) (n=20)
Present	6	2
Absent	14	18
SIGNIFICANCE	p=0.5820	

Bacterial 16S rRNA gene was detected in 31% of the CNNA samples by PCR which were empirically treated with cefotaxime had Dminished PMN count and favourable outcome.

TABLE 25: COMPARING POST TREATMENT PMN DECREASE IN INPATIENTS WITH AFIs (n=124)

AFIs	GPC	GNB	Candida	Total
	26	94	4	124
Post Rx PMN decrease (<25% from baseline)	20	82	2	104

Among the 124 AFIs among inpatients,the PMN count decreased (to <27% from baseline) in 114 cases after treatment with appropriate drug intravenously . The AFIs in which PMN count failed to decrease were due to GNB(12), GPC (6) and candida(2) all of which culminated in death. The mortality rate was 16.3%.

TABLE 26:GROWTH WITH TREATMENT PROFILE,POST Rx PMN COUNT OF INPATIENTS (n=20) WITH AFIs SUCCUMBING TO DEATH (n=20):

AF PMN	GROWTH	Rx	POST Rx PMN COUNT	CAUSE OF DEATH
200	Staph aureus (MRSA)	Vanco1gm iv 12thhrly	200	UGI Bleed/HE
100	CONS (MRS)	Vanco 1gm iv 12thhrly	100	Health care associated pneumonia
300	Escherichia coli (ESBL)	Mero 1gm iv 12 hrly	500	UGIBleed/ Hypovolemic shock
260	Pseudo aeruginosa (ESBL)	Imipenem 500 mg iv thrice daiy	300	UGIBleed/ Hypovolemic shock
100	Enterococcus faecium (VRE)	vancomycin 1gm iv 12thhrly, doxy 100 mg twice daily	100	HE/Aspiration pneumonia/Sepsis
150	Candida albicans	Fluconazole 200mg iv once daily	400	HE/UGI Bleed
20	Pseudomonas species(ESBL)	Pip- tazo 4.5 gm iv 8 hrly	200	Cellulitis leg/Sepsis
50	Pseudomonas species(ESBL)	Pip- tazo 4.5 gm iv 8 hrly	400	UGI Bleed/HE
260	Acinetobacter (MBL)	Imipenem 500 mg iv thrice daily	300	Hepatorenal Syndrome
250	Acinetobacter (ESBL)	Imipenem 500 mg iv thrice daily	200	Hepatorenal Syndrome
AF PMN	GROWTH	Rx	POST Rx PMN COUNT	CAUSE OF DEATH
200	Staph aureus (MRSA)	Vanco1gm iv 12thhrly	200	UGI Bleed/HE
100	CONS (MRS)	Vanco 1gm iv 12thhrly	100	Health care associated pneumonia
300	Escherichia coli (ESBL)	Mero 1gm iv 12 hrly	500	UGIBleed/ Hypovolemic shock

260	Pseudo aeruginosa (ESBL)	Imipenem 500 mg iv thrice daiy	300	UGIBleed/ Hypovolemic shock
100	Enterococcus faecium (VRE)	vancomycin 1gm iv 12thhrly, doxy 100 mg twice daily	100	HE/Aspiration pneumonia/Sepsis
150	Candida albicans	Fluconazole 200mg iv once daily	400	HE/UGI Bleed
20	Pseudomonas species(ESBL)	Pip- tazo 4.5 gm iv 8 hrly	200	Cellulitis leg/Sepsis
50	Pseudomonas species(ESBL)	Pip- tazo 4.5 gm iv 8 hrly	400	UGI Bleed/HE
260	Acinetobacter (MBL)	Imipenem 500 mg iv thrice daily	300	Hepatorenal Syndrome
250	Acinetobacter (ESBL)	Imipenem 500 mg iv thrice daily	200	Hepatorenal Syndrome

All the AFIs which culminated in death of the patients, the post treatment PMN count failed to decrease and all were due to organismsexhibiting special resistance mechanisms. The other co-morbid factors are listed in the table.

DISCUSSION

Ascitic Fluid Infections (AFIs) is the most frequent infection in Cirrhotic patients with prevalence ranging from 9% to 31%. SBP is associated with mortality ranging from 21-41%.

Thorough knowledge of the pathogenesis, risk factors, and the resistance pattern ,organism profile commonly prevalent among them in our setup is necessary to bring down the mortality associated with AFIs.

Leukocyte esterase found in the PMNs andreleased upon lysis of PMNs during inflammatory cascade. The strip when placed inside a test tube of fresh ascitic fluid at the bedside and kept for 90 seconds. The esterase present in the PMNs act on the estersubstrate and releases 3-hydroxy-5-phenyl-pyrrole.this changes the color. The calorimetric scale reference chart is available for direct optical comparison on the sides of the container.

Accuracy	Nochange	Yellow	Blue	Purple
Sensitivity	100%	28.5%	61.4%	100%
Specificity	75%	95.4%	100%	97%

Our results from the LERS strip showed good correlation with Bruce A Runyon[10] mentions sensitivity of strip to be 96%,specificity to be 89% and negative predictive value 99%. Balagopal et a[17]reported sensitivity of 93% and specificity of 98%.

Nitrites also help in diagnosing ascitic fluid infections as it is reduced by the baterias.The sensitivity, specificity, positive and negative predictive are as follows12%, 9%, 41%, and 78%.

But our study showed below the different values of this nitrite reagent test.

ACCURACY	WHITE	PINK	RED	VIOLET
SENSITIVITY	100%	28.5%	61.4%	100%
SPECIFICITY	75%	95.4%	100%	97%

The reactive strips detect nitrite by using the Greiss reaction to produce a pink azo dye.Our results from the nitrite reagent strip showed good correlation withBruce A Runyon[10] mentions sensitivity of strip to be 96%,specificity to be 89% and negative predictive value 99%.

Butani et al[20] used leukocyte esterase reagent strip/nitrite strip test to diagnose SBP in 136 specimens by using grade 2 as a cut-off scale, and found the sensitivity, specificity, PPV and NPV of the leukocyte esterase reagent strip/nitrite strip test as 83%, 99%, 91%, and 98% respectively. In study done by Vanbiervliet et al, nine of 72 patients included were diagnosed with SBP, another leukocyte esterase reagentstrip/nitrite reagent strip test was positive in all cases with 100% sensitivity and specificity.14 Sapey et al found sensitivity, specificity, PPV, NPV of leukocyte esterase reagent strip as 64.7%, 99.6%, 91.7%, and 97.4% respectively.[19] Kim et al revealed 50% sensitivity, 100% specificity, 100% PPV and 87% NPV of the leukocyte esterase reagent

strip/nitrite reagent strip test in his study.[18] Thevenot et al found 89% sensitivity, 100% specificity, 100% PPV and 99% NPV.[17]

Our team evaluated the prevalence of AFIs among 422 Cirrhotics patients withComparison to patient population – outpatients (Op) vs inpatients (Ip), various etiologies, risk factors. Our team also evaluated microbial profile of isolates from Op and Ip population, their antimicrobial susceptibility and screened for special resistance mechanisms exhibited by them. Our team evaluated if bedside inoculation of ascitic fluid into blood culture bottles combined with newer culture methods yielded significant diagnostic yield withrespect to conventional method of laboratory inoculation.

Most of the patients in our study population are of age group 50-61years, followed by 40-51years. The mean age group in the studypopulation is 45.43 years and the median age 47 years. There was a good correlation with the median age in studies by Grunhage et al[5] who70 had median age of 50 years, Dodammani et al[22] with similar most common age group, Zahidullah et al[71] who had population with medianage 52.5 years. Majority were males as expected. (82% of outpatients and91% of inpatients).

The most commonest cause of cirrhosis in the male patients was ALCOHOLIC CIRRHOSIS (66.7% among outpatients and 58.4% among inpatients) which correlated well with study conducted by Pazhanivel[51],Grunhage et al[8]where alcoholic cirrhosis was 57.5% and 64.7%. Cryptogenic cirrhosis was the most frequent cause in cirrhosis

among female inpatients which correlated with the study by Grunhage et al[8].

The majority of outpatients with AFIs were asymptomatic (78.6%).The predominant symptoms among inpatients with AFIs werefever (28.4%) which correlated well with the studies conducted byCaruntu[28](32%) and as stated by McHutchison (68%)[48].

In our by 5.8% of inpatients with AFIs were asymptomatic the importance of these groupwas also as highlighted by Schiff (upto30%)[25].

Leukocytosis was present in only 14% of our study population. Studies conducted by Todd et al[66] also states that there will only be subtleelevation probably due to hypersplenism as stated by Kerr41(11%) andKoulaouzidis[42] in their studies.

The ascitic fluid neutrophil count is increased (>250/mm3) in 108 (67.4%) patients (categorized as SBP) of AFIs in which only 23 patients had elevated peripheral blood neutrophil count. This proved that asciticfluid neutrophil count had no correlation with peripheral blood neutrophil count. This observation in our study proved that the good correlation with the study conducted by Angeloni et al[62]. The PMNs reaches theperitoneal sac with response to specific stimulus and where there are localongoing pathogenesis guided by chemotactic factors (Mainor et al[46]). In this study raised serum total bilirubin (>2.5 mg/dl) was associated with AFIs in 58.5% and this was stastistically significant(p=0.0229) .The importance this parameter as independent risk factor hasbeen ascertained by various studies conducted by

Angeloni et al[62], Riberiet al[55] and Jiannis Anastasiou[34] who confirmed the importance in their
multivariate analysis.

Low serum albumin level (<3.5 g/dl) is present with AFIs in 91.4% of our study. Serum albumin level also forms a good variable in CTP score. The significance of this parameter as risk factor is published in article published by Erica Horinek[24]and but we see no Significant stasistical association (p=0.2624) in our study.

The second most symptom among inpatients is UGI bleeding (23%). The prevalence of AFIs among cirrhotic patients with UGI bleeding was 69.6% in our study which showed good correlation with the study done by Yang et al [70] who states 66% association. Thalimer et al[65]and Goulis et al[30] also reports 66% prevalence 50% respectively. Thisassociation was highly stastistically significant (p=0.0002)

AFIs were prevalent in 88% of patients with HRS-I and 65.7 % of patients with HRS-II in our study. Raised serum creatinine (>1.1 mg/dl)was found as an independent predictor of mortality in study conducted byLubna Kamani[44] and Anastasiou et al[34] in their multivariate analysis. This association was of high stastistical significance (p=0.0098) .

In our study the cause of death was concluded as HRS in 4 of the patients.Most of the outpatients belonged to Class B (73.4%) ,inpatients belonged to Class C (68.2%) in our study. AFIs prevalence in DCLD patients belonging to CTP Class C (63%). This correlates well the

review article by Cesar Alaniz[20] who states 72% SBP occurs in CTP Class C.Analysis by Anastasiou et al34 and Such et al37 also stated this. In our study no patients belonged to Class A and *nosignificant difference (p=0.0879)* among prevalence of AFIs between Class B and C patients.

In our study inpatients 52.5% with high score (>15) and 48.5% withlow score (<15). The AFIs was of equal prevalence in DCLD patientswith high (48.8%)and low (51.2%) MELD scores *(p=0.1529).*The AFI was associated with acase of Whipple's procedure, a case of malignant ascites, and a case each of Wilson's Disease, NonAlcoholic Fatty Liver Disease, Non Cirrhotic Portal Fibrosis, HBV+SOLRt.lobe of Liver (?HCC) supported by Isner[32] who also reported aninteresting case of gastric CA metastasis associated with SBP and 2 cases of Cholestatic jaundice and Budd-Chiari Syndrome. Person et al. havereported two patients with Wilson's disease who developed SBP[52].

It was pointed out by Todd[47] in his review article that SBP occurrence in other causes of cirrhosis is rare. Hepatocellular carcinoma may result in anincrease in the risk of developing SBP in cirrhotic patients by furtherimpairing antimicrobial defense mechanisms (Goulis et al[30]).

The (p value=0.4271) had no change , so we conclude that patientswith any etiology to be screened for AFI by ascitic fluid culture.We found that the prevalence of AFIs was 25% among outpatients most of them are asymptomatic and in inpatients is 44.3%

and that difference ishighly statistically significant (p=0.006).This correlated well with Todd A.Sheer66 who had prevalence of 30% and 3.5% respectively. Also had good correlation with other studies by Bunchorntavakul et al[21], . Studies by Vargese et al[69], Evans et al[45], Pazhanivel[51], Riberio et al[55] AFIs among outpatients were found to be 3.7% ,3.5% , 2.5% and 11% respectively.Lubna Kamani[44],carnutu et al[28] showed 25-31%, 24% and 25% prevalence among inpatients respectively.

In our study 76% of AFIs in outpatients are due to Gram positive organisms and 76.8% of AFIs are inpatients, due to gram negatives and this difference was highly stastistically significant*(p=0.005)*and this had good correlation with Riberio et al55 where 80% was due to GNB and as stated by Rimola et al5(65%).This is due to the quinolone prophylaxis and emergence of AFIs by gram positives among outpatients and drug resistant GNB emergence, leading to complications needing hospitalisation.

There was two case of Tuberculous Peritonitis. There was no lymphocytic predominance as supported by Faisal M.Sanai[26]. But LJ medium culture yielded growth in 4 weeks in ourstudy, done after centrifugation of one litre of ascitic fluid as supportedby Mimidis et al[49], Ali Uzunkoy[2] (6 weeks). The Tubercle bacilli was absent in AFB Smear as supported by Angeline et al[4], Ali Uzunkoy[2],Kashyap et al[53], Mimidis et al[49](3%), BruceA.Runyon (AFB SMEAR-0% SENSITIVITY) who said that thesensitivity of AFB smear is very low and usually AFB smear isnegative.

Mimidis et al[49]found positive culture in 22% of cases. Rapid results (14 days) with sensitivity (sensitive to rifampicin and INH) were obtained by MGIT (Tsi-ShuHuang et al[67])

In our study, on ascitic fluid PMN count. 108 (66.4%) were categorized as SBP and 50 (32w.6%) were categorized as MNB, Dodammani et al[22]had 89% SBP and 14% MNB among culture positiveAFIs. GPC was predominant among the MNB category and GNB waspredominant among the SBP category and this was highly statisticallysignificant (p = 0.0004). This showed good correlation with Riberio etal[55], Dodammani et al[22] who found 81% and 63.5% of AFIs due to GNBamong SBP.20 of our patients had CNNA.

In our study, Direct gram stain (DGS) organisms were seen only in 24 samples which are macroscopically cloudy/slightly turbid. Atleast 10^5 organisms/ml in the sample need to be present to be seen by DGS. The median ascitic concentration of bacteria inAFIs was1 organism/mL. Expecting to see organism in DGS of ascitic fluid was as bad as searching bacteria from Gram stain of blood(sleisenger[61]), also supported by Cesar Alaniz[20]-poor diagnostic yield.

In our study, conventional method found around 23.8%,Triton-X Found 39%, bedside inoculation Found 88.6%, and bedside inoculation combined with 2% Tween-80 BAP Found 100% of culturepositives. Each of the other three methods Found more culture positiveswith respect to conventional laboratory inoculation and there was highstatistical significance for all the three methods (p=0.000) incomparison with conventional method. This was due to

lowconcentration of bacteria (1-2 organisms/ml) , delay in transport which lead to continuous phagocytosis and the recovery was low,(Runyon[15]).

So small variations in cultures processing has hugeimpact on the results like bedside inoculation which diluted the effects ofantibodies, complement,previously used antibiotics as also concluded bySumittra Charoenhirunyingyos[63].

Klebsiella, the common isolate (30.2%) followed by Escherichia coli(12.7%)among GNB. Cesar et al[20] in his study stated that 46% of AFIs were due to Escherichia coli and Klebsiella accounted for 9%. Caruntu et al[28]observed 60% were due to Escherichia coli and Klebsiella put together.

Among GPC the common isolate was Staphylococcus aureus (12.9% of AFIs). Overall GNB isolated was 77% and GPC was 22% and candida 4%.

In our study, the 9 organisms additionally recovered using of 2% Tween 80 BAP were Klebsiella pneumoniae(2) Enterobacter(1) Citrobacter freundii(1), Pseudomonas aeruginosa(1) and Pseudomonasluteola(2) and Acinetobacter baumanii(2) among which 3 were ESBLproducers and one carbapenemase producer.

In our study, obligate anaerobes were not isolated from any of the samples. This showed good correlation with study by Cesar et al[20] whoreported 0%. This was in accordance that the anaerobes

thoughpredominant in bowel flora are unable to translocate and unable toproliferate in ascitic fluid with high oxygen content. (Carnutu et al[28]and Cesar et al[20] and Todd A.Sheer[66]).

In our study among patients with AFIs, glucose level was<50mg% in 67.6% and in the range 50-100mg% in 27.1%respectively. This observation was of high statistical significance(p=0.0001). Normally, the concentration of glucose in ascitic fluid is parallels that of seum. In bacterial infection of ascitic fluid, the glucose drops down and sometimes even upto as stated by (Sleisenger[61]).

AF albumin levels <1.5g/dl with highest incidence of AFIs as pointed by Kashani A et al[40], Anastasiou et al[34]. In our study,prevalence of AFIs in with this AF albumin <1.5g/dl was 88% and AF total protein 1.5g/dl was 52%. The former association wasstatistically highly significant(p=0.0001), while the latter associationwas statistically significant (p=0.0217). This showed good correlation with Schiff [25](24%).

The prevalence of MRS, MBL ,ESBL, HLAR ,VRE, and among pathogens responsible for AFIs among inpatients were 67.6%,41.4%,28%,29% and 2.8% respectively.

In GNB norfloxacin resistance was 62.7% and 59.5% among staph species. In GNB Ciprofloxacin resistance was 27 % and 23.8% among staph species. The prevalence of MRS and ESBL among pathogens responsible for AFIs among outpatients were 12.9%

(p=0.0499) and 21% (p=0.6375) respectively. Among GNB norfloxacinresistance was absent in isolates from outpatients and 59% amongStaph species.

Norfloxacin resistance was widespread in isolates from inpatients than outpatients and this was highly statistically significant (p=0.003), while isolates from both group of patients showed decresed resistance to ciprofloxacin (p=0.373).

We did an interesting observation in our study: Among the 52 patients with Hepatic Encephalopathy,AFIs were positive for 36 patients and among them UREASE producing organisms were 30 (84.3%) .This observation was highly statistically significant (p=0.0078).

Among the inpatients with AFIs with bacteremia as evidenced by positive blood cultures is seen in 28 (22.5%). Bert et al12 study showed 24.5%. Gram negatives were isolated in 20 (71.4%), gram positives in 2 and candida in 4 (14.3%) cases respectively in our study. Anastasiou et al[34] observed candidal sepsis in 15% of AFIs. About 12 (42.9%) patients with bacteremic AFIs succumbed to death (2 GPC+8 GNB+2 Candida). Shizuma et al[60] had mentioned in her articlethat bacteremic AFIs were common in patients with UGI bleeding andin our study we found positive association 20 patients (72.4%) had h/oUG Bleeding. 12 (43.9%) patients with bacteremic AFIs succumbed to death.

Bactrial DNA (byPCR amplification of 16S rRNA) was present in three ascitic fluid samples from 20 patients with CNNA compared with 20 CNNNA samples. In our study, the culturenegativity in these patients could be explained by continuous ongoingopsonisation process so that only remains of bacterial wall or innercomponents only will be found in ascitic fluid that could not be revivedby culture (Such et al[38]).CNNA is the expected 20% failure rate toisolate the organism (Hecser et al[31]).All the CNNA patients were routinely treated with favourable outcome (p=0.5820).

Mostafa et al[47] reported Sughira et al study were 11.4% of CNNA patients had bacterial DNA. This was consistent with the fact that BACTERIALTRANSLOCATION ACROSS gut that is important in pathogenesis ofAFIs.

Among the 124 AFIs among inpatients, the PMN count decreased (to <25% from baseline) in 108 cases after treatment with appropriate drugintravenously . The AFIs in which PMN count failed to decrease were due to GNB(12), GPC (6) and candida(2) all of which culminated in death.

The mortality rate was 16.2% in our study. Lubna Kamani[44]reported 30% mortality .We put forth that the use of bedside inoculation into blood culture bottles combined with newer culture detection methods employing non-ionic surfactants decreased the turn around time which was responsible for bringing down the mortality rate.

All the AFIs which culminated in death of the patients, the post treatment PMN count failed to decrease and all were due to organisms exhibiting special resistance mechanisms like ESBL, Carbapenemaseproduction and GPCs Methicillin Resistant Staph AND Vancomycinresistant enterococci were seen. This showed good correlation asobserved by Kyoung-Ho Song[43]. The other co-morbid factors present among patients with AFIs that culminated in death were UGI bleeding(6), UGI bleeding AND HE (4) HRS(4)HE(4),Health care associated pneumonia(2).

SUMMARY

In the cross sectional study conducted among 422 DCLD patientsstated that LER strip test and nitrite reagent strip test had better accuracy with increased specificity of 96% and sensitivity of 89%,PPV 94% NPV 98% and accuracy of 100% for SBP. Grade II /IIIcolor had better indicators of SBP.These two grades used are 125 and 500 PMNL/ mL in comparison to the gold standard for diagnosis of SBP of 250 PMNL/mL. These two values were useful for identifying SBP.

The LER is according to the esterase activity of granulocytes. These cells release leukocyte esterase into the extracelullar milieu reacts with an ester releasing 3-hydroxy-5-phenyl-pyrrole which causes a color change in the azo dye in the reagent strip.

The strip in our study accurately identified PMN count in the lowest range (0- 70) and the highest range (>500) with appropriate colour change, that is, with 100% accuracy .For the PMN counts in the ranges (71-125) and (126-500) the colour change was appropriate in 28.5% and 61.4% respectively.

Results of this study are quite different from otherstudies published to date evaluating the use of leukocyte esterase dipstick test in the diagnosis of SBP as this study not only detects the SBP bedside but also compares the Bed side inoculation of ascitic fluid in culture bottle and also identify the organism responsible for the infection and follow

up bed side LER strip test with ascitic fluid bed side inoculation showing reduction in cellcount after antibiotic therapy.

Vanbiervliet *et al* had first studied the use of LER in Ascitic Fluid. They studied using *Multistix 8 SG* strips,and
the sensitivity and specificity was 100% .
The sensitivity for the diagnosis of SBP has ranged between 85% and 100%, and the specificity between 98% and 100%.

Thus,strip test is accurate, rapid, easy and cheaper. Further, it should be possible to apply this test to determine the effectiveness of antibiotic therapy in patients with SBP by repeating the test, though this aspect needs further studies.

Castellote et al[15] studied the use of Aution sticks (A. Menarini Diagnostics, Firenze, Italy) for diagnosis of SBP in cirrhotic patients with ascites who underwent abdominal paracentesis at a university based hospital, and found that the sensitivity, specificity and PPV are 89%, 99% and 98%, respectively. The benefit of the Aution stick is the precise colorimetric scale that correlates with ≥250 PMN cells/mm3. Sarwar et al used Combur 10 urine strip fordiagnosis of SBP and found 97.7% sensitivity and89.4% specificity with positive predictive value of 90%, negative predictive value of 97.7%.[27]

The limitations of the strip test include absence of a cut-off corresponding to cell count of 250 PMNL/mL in the reagent strip, and the possibility of inter-observer variation in matching of color.

In the cross sectional study conducted among 422 DCLD patients the prevalence of AFIs was 38.6%. The prevalence of AFIs among DCLD patients with CIRRHOSIS and Mixed ascites (Cirrhosis plus another cause for ascites) was 78%.The prevalence of AFIs among DCLD of other etiologies-Cryptogenic causes, Budd-Chiari Syndrome, Cholestatic jaundice, Wilson's Disease, Non Alcoholic Fatty Liver Disease, Non Cirrhotic Portal Fibrosis, Hepatocellular carcinoma, Post Whipple's procedure (Hypoalbuminemia) was 28%.So,invariably in patients with non-cirrhotic ascites (because of other etiologies also) we recommend ascitic fluid culture be done as admission surveillance tap.

The prevalence of AFIs was 47.3% among inpatients and 28% among outpatients with DCLD. Yet the prevalence rate was higher in our study compared with other studies. So, we would like to emphasise that ascitic fluid culture should be performed in all cases of undiagnosed ascites attending the op, and have constant vigilance over patients on therapeutic paracentesis.

From the results of our study we conclude that bedside inoculation of ascitic fluid into blood culture bottles combined with newer culture methods using non-ionic surfactants yielded 77.3% higher culture positivity than conventional method.

Overall 75.9% of the AFIs were due to gram negative bacilli, 23% due to gram positive cocci and 3.3% due to candida. Among the inpatients gram negatives were the predominant cause (75.9%) and among outpatients gram positives (78%) were the predominant cause of AFIs respectively. The prevalence of Methicillin Resistant

Staphylococcus (MRS) and Extended Spectrum Beta Lactamase producers (ESBL) were 12.6% and 21% among outpatients & 66.6% and 40.3% among inpatients respectively. Among inpatients the prevalence of VRE , HLAR and carbapenemase producers was 26%, 26% and 2.4% respectively.

The GNB and GPC resistant to norfloxacin among those isolated from outpatients were 0% and 57% respectively. Among the GNB and GPC isolated from inpatients the prevalence of norfloxacin resistance was 61.4% and 69% respectively.

We would like to put forth that this clone of drug resistant gram positives and gram negatives exacerbates the ongoing pathogenesis in these patients who are already immunocompromised leading to complications that require hospitalisation, risk of future episodes of AFIs and other septic complications.

We concluded from the results of our study that combining bed side strip testing of ascitic fluid and bedside inoculation into blood culture broths with newer techniques decreased the number of ascitic fluid samples as being falsely reported sterile and early, specific antibiotic therapy was given to the patients.

CONCLUSION

In conclusion, our study shows that LER strip test has Excellent accuracy for SBP, and it is having high speed, less cost, easy to use, and doesn't require expert hands. Because of its speed, the use of this test may help early institution of antibiotic therapy in patients with SBP.

The prevalence of AFIs among DCLD patients with cirrhosis was 79% and the prevalence among patients with various other etiologies for DCLD (like Cryptogenic, Budd Chiari Syndrome, Hepatocellular carcinoma etc) was 28%.

The prevalence of AFIs was 26% among outpatients and 44.7% among inpatients with DCLD.

Klebsiella was the most common isolate (30.4%), followed by Escherichia coli (12.6%) and Staphylococcus aureus (12.8%). Gram positive cocci (77%) were the predominant cause of AFIs among outpatients and Gram negative bacilli (75.9%) were predominant cause of AFIs among inpatients.

The prevalence of Methicillin Resistant Staphylococcus (MRS) and Extended Spectrum Beta Lactamase producers (ESBL) were 12.5% and 20% among outpatients & 66.5% and 40.4% among inpatients respectively.25% of Enterococci isolated among inpatients exhibited Vancomycin Resistance (VRE) and High Level Aminoglycoside

Resistance (HLAR).The prevalence of carbapenemase production was 2.1% among isolates from inpatients.

The percentage of resistance to norfloxacin among GNB and GPC isolated from outpatients was 0% and 50% & inpatients was 61.7% and 67% respectively.

The percentage of resistance to ciprofloxacin among GNB and GPC isolated from outpatients was 20% and 25% & inpatients was 29.8% and 22.2% respectively.

APPENDIX

Ascitic fluid test strip

Leukocytes

Nitrite

Urobilinogen

Protein

pH

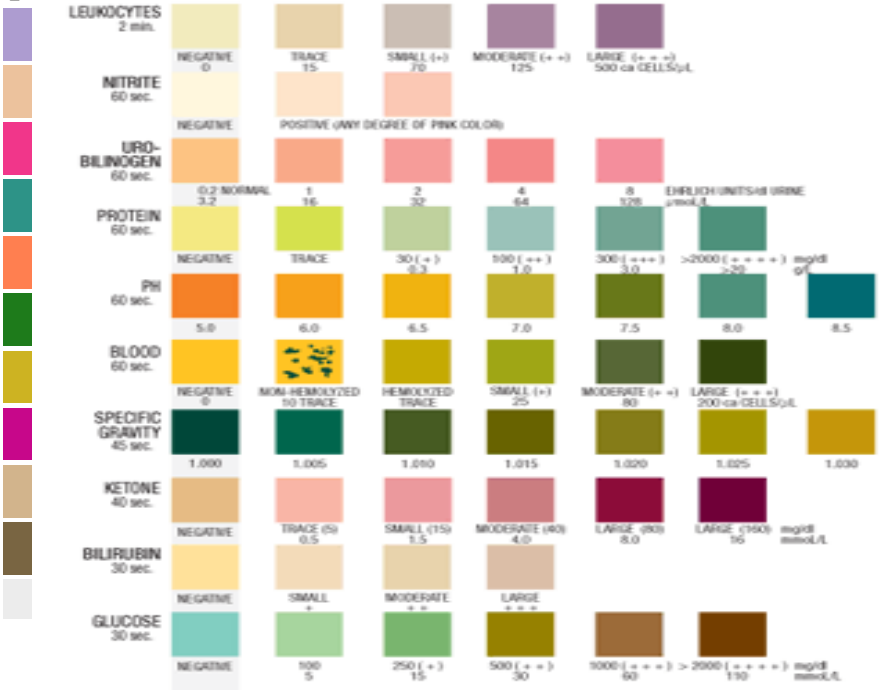
Haemoglobin

Specific gravity

Ketone

Bilirubin

Glucose



LERS TEST

It is normal so find up to 3 (occasionally 5) leukocytes per high power field (40X) in a ascitic fluid sample. The strip test for white blood cells detects leukocyte esterase, which is present in azurophilic granules of monocytes and granulocytes (neutrophilic, eosinophilic and basophilic). Bacteria, lymphocytes and epithelial cells from the genitourinary tract do not contain esterases. Neutrophil granulocytes are the leukocytes most commonly associated with ascitic fluid infections.

A positive test for leukocyte esterase normally indicates the presence of bacteria and a positive nitrite test .

Theasciticfluid test strip reaction is based on the action of leukocyte esterase in catalysing the hydrolysis of an ester of indolecarboxylic acid. The indoxyl that is liberated combines with a diazonium salt in order to produce a violet coloured azole dye.

- 1) Reaction catalysed by leukocyte esterase

  Indolecarboxylic acid ester → Indoxyl + Acid

- 2) In acid medium

  Indoxyl + Diazonium salt → Violet azole dye

The esterase reaction needs about 5 minutes to take place. The presence of strong oxidising agents or formaldehyde can cause false positives. False negative results are associated with elevated concentrations of protein (greater than 500 mg/dL), glucose (greater than 3 g/dL), oxalic acid and ascorbic acid.

NITRITES TEST

The test for nitrites is a rapid screening method for possible asymptomatic infections caused by nitrate-reducing bacteria.. The test is a rapid screen for possible infections by enteric bacteria

The reactive strips detect nitrite by using the Greiss reaction in which the nitrite reacts in an acid medium with an aromaticamine (para-

arsanilic acid or sulphanilamide) in order to form a diazonium salt that in turn reacts with tetrahydrobenzoquinoline to produce a pink azo dye.

- 1) In an acid medium

  Para-arsanilic acid or sulphanilamide + NO_2 → Diazonium salt

- 2) In an acid medium

  Diazonium salt + tetrahydrobenzoquinoline → Pink azo dye

BRAIN HEART INFUSION BROTH

Sodium citrate 1 gm
Sodium chloride 4 gm
Sodium phosphate 5 gm
Dextrose 10 gm
Peptone 10 gm
Brain infusion broth 250 ml
Heart infusion broth 750 ml

Obtain ox brain and heart. Remove all fat from the heart. Cut into small pieces and grind. Add distilled water three times. Keep at 4°C overnight. From the brain remove meninges fully and weigh. Add distilled water three times. Keep at 4°C overnight. Next morning boil the brain and heart separately, for 30 minutes.

Then, filter through cotton layer. Mix both infusions and the remaining ingredients. Dissolve well and adjust pH of to 7.4 -7.6. Autoclave at 121°C for 15 min. Filter through filter paper and distribute about 50 ml in screw capped bottles .Autoclave once more at 115°C for 10 minutes.

THIOGLYCOLLATE BROTH

Yeast extract, water soluble 5 g
Casein hydrosylate, pancreatic digest 15g
Glucose 5.5 g
L-cystine 0.5 g
Agar 0.75 g
Sodium chloride 2.5 g
Sodium thioglycollate
(mercaptoacetate) 0.5 g
Resazurin sodium solution,1 in 1000,
freshly prepared 1ml
Water 1 litre

Dissolve the ingredients other than thioglycollate and resazurin by steaming at 100°C. Add the thioglycollate and adjust the pH to 7.3.If there is a precipitate, heat without boiling and filter hot through moistened filter paper. Add the resazurin solution, mix thoroughly, distribute and sterilize by autoclaving at 121°c for 15 minutes. Cool at once to 25 °c and store in the dark between 20°c and 30°c.If more than the upper third is pink in colour, anaerobic conditions may be restored once only by steaming at 100°c for a few minutes.

BLOOD AGAR

The medium is prepared by adding sterile defibrinated sheep blood(5-10%) to sterile nutrient agar that has been melted and cooled to 50°c.Mix well and pour plates. Any bubbles caused by mixing of the blood and the agar can easily be removed bydrawing a Bunsen flame quickly across the surface of the medium in the dish.

MAC CONKEY AGAR

Peptone 20g
Sodium taurocholate 5g
Agar 20 g
Neutral red solution,
2% in 50% ethanol 3.5g
Lactose, 10 % aqueous solution 100ml
Dissolve the peptone and taurocholate in the water by heating. Add the agar and dissolve it in the steamer or autoclave. If necessary, clear by filtration. Adjust the pH 7.5.

2% TWEEN 80 BLOOD AGAR

Constituents-Tween 80 (2%; 20 ml) incorparating Columbia agar base (39.0 g; Oxoid), distilled water (910 ml), and sheep blood (7%; 70 ml) (Difco) was prepared by the following method. The agar was mixed, and the Tween 80 was added. The mixture was autoclaved at 121°C for 15 min and allowed to cool, the blood was added, and the plates were poured. Plates were kept at 4°C for up to 4 weeks.

CHOCOLATE AGAR(HEATED BLOOD AGAR)

Sterile defibrinated blood 10 ml
Nutrient agar(melted) 100 ml
Melt the nutrient agar. When the temperature is about 45 to 50 °C add the blood and mix well. After the addition of blood, heat in water bath slowly bringing up the temperature to 75°C with constant agitation. Heating is continued till the blood changes to chocolate colour. Cool to about 50°C and pour plates.

BRAIN HEART INFUSION AGAR

Agar granules 1.5 gm
Brain Heart infusion broth 100 ml
Dissolve agar and autoclave it at 121°C for 15 min.Cool to about
50°C and pour plates.

MUELLER- HINTON AGAR

Beef infusion 300 ml
Casein hydrosylate 17.5 gm
Starch 1.5 gm
Agar 10 gm
Distilled water 1000 ml
The ingredients were dissolved in one liter of distilled water, mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. pH adjusted to7.4. Sterilized by autoclaving and poured in plates.

SABOURAUD DEXTROSE AGAR

Dextrose 40 gm
Neopeptone 10 gm
Agar 20 gm
Distilled water 1000 ml
Mix the ingredients in water by heating. Adjust the PH to 5.6.Sterilize in autoclave at 121°C for 15 min. Cool 100 ml of sterile SDA to 50°C.Add 5 mg of Chloramphenicol, dissolved 1ml of 95% alcohol and gentamicin. Mix and pour plates.

LOWENSTEIN JENSEN MEDIUM

BUFFERED SALT SOLUTION
Potassium dihydrogen orthophosphate 28 gm
Disodium hydrogen orthophosphate 16 gm
Magnesium sulphate 1.6 gm
Citric acid 12 gm
L-Asparagine 40 gm
Glycerol 80 ml
Distilled water 4 litres.
Add and dissolve the ingredients. Dispense in 400 ml amounts and autoclave at 110°C for 10 min.

COMPLETE MEDIUM

Buffered salt solution 1200 ml

Whole egg 2000 ml

1% M alachite green 80 ml

Scramble the eggs using a mixer and add the other reagents. The PH of the medium is 6.95.Dispense in 3.5 ml amounts in thin glass universal containers. Inspissate containers horizontally at 85°C for 1 hour to form a solid slope.

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KEY TO MASTER CHART

Cr - Seru m Creatinine

AF - Ascitic Fluid

AF-TC - A scitic Fluid Total Count

AF-PMN - Ascitic Fluid Polymorphonuclear leukocyte count

LERS - Leukocyte Esterase Reagent Strip

NC - No change

AF-alb - As citic Fluid albumin

SAAG - Serum Ascites Albumin Gradient

Hb - Haemoglobin

TC - Total white blood cell count

DC - Differential white blood cell count

P - Polymorphs

L – Lymphocytes

Ab pain - Abdominal pain

UGI bleeding - Upper Gastrointestinal b leeding

HE - Hepatic Encephalopathy

CTP - Child Turcotte Pugh Score

MELD - Model for End- Stage Liver Disease Score

Bilirubin(Tot/Dir) - Total and Direct blirubin

AST/SGOT - Serum aspartate aminotransferase/Serum glutamateoxaloacetate transaminase

ALT/SGPT - Serum alanine aminotransferase/Serum glutamate pyruvatetransaminase

ALP - Serum alkaline phosphatase

PT - Prothrombin time

INR - International normalised ratio

R - Monocytes, Eosinophils, Basophils

DGS - Direct gram stain

Gen - Gentamicin 10 µg

Ak - Amikacin 30 µg

Amc - Amoxyclauv 10/30 µg

PTZ - Piperacillin Tazobactam 10/100 µg

HLG - High Level Gentamicin 120 µg

Van/Vanco - Vancomycin 30 µg

Tetra - Tetracycline 30 µg

Imi - Imipenem 10 µg

WM - Wet mount

Org - Organism

AFB - Acid Fast Bacilli

BHI - B rain Heart Infusion broth

BAP - Blood agar plate

SDA - Sabouraud's Dextrose Agar

Post Rx PMN - Post Treatment Polymorphonuclear leukocyte count

ND - Not done

NG - No growth

ANTIBIOTIC DISCS

Cefo - Cefotaxime 30 µg

Cx - Cefoxitin 30 µg

Cefta - Ceftazidime 30 µg

Nor - Norfloxacin 5 µg

Cip - Ciprofloxacin 5 µg

Pen G - Penicillin G 10 Units

Mero - Meropenem 10 µg

MRSA - Methicillin Resistant Staphylococcus aureus

MSSA - Methicillin Sensitive Staphylococcus aureus

ESBL - Extended Spectrum beta Lactamase

MBL - Metallo beta Lactamase

VRE - Vancomycin Resistant Enterococci

MASTER CHART

Name	AGE	SEX	OP/IP	Alcoholic	HBV	HCV	OTHERS	Ab Pain	UGI Bleedi	Diarrhoea	HE	Fever	CTP	MELD	BILIRUBIN(	BILIRUBIN	AST/SGOT	ALT/SGPT	ALP	PT	INR	Tot Proteir	Albumin	Globulin	RFT UREA
MARIAMM	45	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	11/C	12	2.5	1.2	21	14	355	14	1	4.1	1.9	2.2	16
GOPALAKR	57	MALE	OP	YES	3.8 IU/ML	NEG		NO	NO	NO	NO	NO	8/B	10	1.22	0.45	43	29	70	14	1	7.1	2.5	4.6	29
MOHAN	52	MALE	OP	YES	NEG	NEG		YES	NO	NO	NO	NO	7/B	6	1	0.2	36	25	40	14	0.9	7	4.5	2.5	16
JOTHI	58	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	8	0.54	0.52	36	24	19	17	1.2	6.3	1.7	4.6	46
SUBBULAK	45	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	15	1	0.2	43	140	100	28	2.2	5.5	3.2	2.3	27
KAMARAJ	40	MALE	OP	NO	NEG	36714 IU/ML		NO	NO	NO	NO	NO	8/B	14	3	1.5	40	40	100	15	1.3	7.3	2.7	3.7	18
MARIAL	45	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	6	0.23	0.1	21	14	355	14	1	4.1	1.9	2.2	16
KANAGARA	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	9	1	0.2	39	19	115	14	1	5	2.9	2.1	37
SHANKARA	53	MALE	OP	NO	NEG	NEG	NAFLD	NO	NO	NO	NO	YES	11/C	14	4.5	2.5	74	75	100	14	1	3.5	2.5	1	46
SENTHIL KI	34	MALE	OP	YES	NEG	NEG	ACUTE PAI	NO	NO	NO	NO	YES	7/B	6	0.9	0.4	37	16	149	14	1	7.3	4.9	2.4	34
SUNDAR R.	43	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	7/B	8	0.9	0.4	45	44	134	14	1	6.1	3.2	2.9	38
LOGESH	36	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	6	0.6	0.2	37	28	130	14	1	5.7	3.2	2.5	27
BALAJI	44	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	6	0.4	0.2	36	27	140	13	0.9	5.6	3.1	2.5	22
LAKSHMAI	27	MALE	OP	YES	NEG	NEG	ACUTE PAI	NO	NO	NO	NO	NO	7/B	6	0.6	0.2	59	36	310	14	1	5.4	3.6	1.8	15
VENKATAC	54	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	7/B	9	2	0.8	78	82	250	14	0.9	5	3.5	1.5	36
ELUMALAI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	9	2	0.6	76	78	180	14	0.8	4.5	2.8	1.7	36
SHANKAR	42	MALE	OP	YES	248IU/ML	NEG		NO	NO	NO	NO	NO	10/C	16	8.5	6.8	84	78	230	16	1.1	7.6	3.1	4.5	6
JAFFER	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAJA	45	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
MARAN	45	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
KUMARESA	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	28	2	0.8	78	64	240	21	2	6.2	3	3.2	33
MARIAMM	54	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	10/C	15	2.6	1.4	34	38	100	16	1.3	3	2	1	34
SUBRAMAI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
RAJAN	36	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
SOMU	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	12	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
GOVINDAR	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	9	2	0.6	76	78	180	14	0.8	4.5	2.8	1.7	36
ADAM	42	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	16	8.5	6.8	84	78	230	16	1.1	7.6	3.6	4	6
VENKATES	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	28	2	0.8	78	64	240	21	2	6.2	3	3.2	33
RAJESWAR	38	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	7/B	6	0.8	0.4	18	21	88	13	1	7.2	4.6	2.6	28
BALAJI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	11	2.2	1.6	96	84	210	14	1	4.5	2.5	2	36
ARUN KUN	24	MALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	YES	9/B	15	2.4	1.6	22	16	60	12	1	6	2.7	2.3	65
PUGAZHEN	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
CHANDRAS	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SRINIVASA	44	MALE	OP	NO	NEG	NEG	NCPF	NO	NO	NO	NO	NO	10/C	16	3.7	1.5	72	32	527	20	1.5	6.5	3.1	3.4	14
MAHESWA	38	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	7/B	6	0.8	0.4	18	21	88	13	1	7.2	4.6	2.6	28
SELVARAJ	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAMAN	54	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
BALAMURI	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SUJATHA	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
MOHAN	52	MALE	OP	YES	NEG	NEG	?MIXED	NO	NO	NO	NO	NO	7/B	8	1.4	0.6	23	190	660	13	1	4.5	4.2	0.3	28
KALA	20	FEMALE	OP	NO	NEG	NEG	?TB,?MALI	NO	NO	NO	NO	NO	8/B	10	2.4	1.6	22	10	73	14	0.8	5.9	4	1.9	37
KOTESWAI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SIGAMANI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
KRISHNAN	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SHADIK BA	34	MALE	OP	NO	NEG	NEG	BUDD CHI	NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
JOTHI	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
SAMBATH	30	MALE	OP	YES	NEG	NEG		YES	NO	NO	NO	NO	8/B	7	1.2	0.2	40	36	70	14	1	5	3.5	1.5	26
VIMALA	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
GOPI	28	MALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	10	1.28	0.23	107	53	245	16	1.3	7.1	2.5	4.6	27
MURUGAN	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
JOHN BASI	44	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	11/C	17	4	2.6	88	98	230	12	1	4.5	2.7	0.7	34
SARALA	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
KRISHNAM	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
VARADARA	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SRINIVASA	75	MALE	OP	NO	22,400,00(	NEG		NO	NO	NO	NO	NO	12/C	29	2.8	2	52	35	100	13	1	4.5	3	1.5	34
BABU	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SUNDARAL	45	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	15	10.3	6	57	28	57	13	1	6.7	3.9	2.8	22
MUNNUSA	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAMU	39	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	12/C	25	12.4	9.8	88	57	305	26	2.2	6.2	2	4.2	46
JALENDRAI	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
ALAGESAN	36	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAMAKRIS	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
BABU	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
KRISHNAN	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	14	2.8	0.68	74	213	220	14	1	7.1	2.5	4.6	29
RAJU	42	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	16	8.5	6.8	84	78	230	16	1.1	7.6	3.1	4.5	6
BASKAR	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30

MASTER CHART

RAJU	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	14	2.8	0.68	74	213	220	14	1	7.1	2.5	4.6	29
LINGADUR	42	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	16	8.5	6.8	84	78	230	16	1.1	7.6	3.1	4.5	6
RAMACHA	54	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	16	8.5	6.8	84	78	230	16	1.1	7.6	3.1	4.5	6
MUNUSAM	30	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.2	40	36	70	14	1	5	3.5	1.5	26
MOHAN	52	MALE	OP	YES	NEG	NEG	?MIXED	NO	NO	NO	NO	NO	7/B	8	1.4	0.6	23	190	660	13	1	4.5	4.2	0.3	28
KALA	20	FEMALE	OP	NO	NEG	NEG	?TB,?MALI	NO	NO	NO	NO	NO	8/B	10	2.4	1.6	22	10	73	14	0.8	5.9	4	1.9	37
KOTESWAI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SIGAMANI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
MARIAMM	45	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	11/C	12	2.5	1.2	21	14	355	14	1	4.1	1.9	2.2	42
GOPALAKR	57	MALE	OP	YES	3.8 IU/ML	NEG		NO	NO	NO	NO	NO	8/B	10	1.22	0.45	43	29	70	14	1	7.1	2.5	4.6	29
MOHAN	52	MALE	OP	YES	NEG	NEG		YES	NO	NO	NO	NO	7/B	6	1	0.2	36	25	40	14	0.9	7	4.5	2.5	16
JOTHI	58	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	8	0.54	0.52	36	24	19	17	1.2	6.3	1.7	4.6	46
SUBBULAK	45	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	15	1	0.2	43	140	100	28	2.2	5.5	3.2	2.3	27
KAMARAJ	40	MALE	OP	NO	NEG	36714 IU/ML		NO	NO	NO	NO	NO	8/B	14	3	1.5	40	40	100	15	1.3	7.3	2.7	3.7	18
MARIAL	45	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	6	0.23	0.1	21	14	355	14	1	4.1	1.9	2.2	16
KANAGARA	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	9	1	0.2	39	19	115	14	1	5	2.9	2.1	37
SHANKARA	53	MALE	OP	NO	NEG	NEG	NAFLD	NO	NO	NO	NO	YES	11/C	14	4.5	2.5	74	75	100	14	1	3.5	2.5	1	46
SENTHIL KI	34	MALE	OP	YES	NEG	NEG	ACUTE PAI	NO	NO	NO	NO	YES	7/B	6	0.9	0.4	37	16	149	14	1	7.3	4.9	2.4	34
SUNDAR R	43	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	7/B	8	0.9	0.4	45	44	134	14	1	6.1	3.2	2.9	38
LOGESH	36	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	6	0.6	0.2	37	28	130	14	1	5.7	3.2	2.5	27
BALAJI	44	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	6	0.4	0.2	36	27	140	13	0.9	5.6	3.1	2.5	22
LAKSHMAI	27	MALE	OP	YES	NEG	NEG	ACUTE PAI	NO	NO	NO	NO	NO	7/B	6	0.6	0.2	59	36	310	14	1	5.4	3.6	1.8	15
VENKATAC	54	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	7/B	9	2	0.8	78	82	250	14	0.9	5	3.5	1.5	36
ELUMALAI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	9	2	0.6	76	78	180	14	0.8	4.5	2.8	1.7	36
SHANKAR	42	MALE	OP	YES	248IU/ML	NEG		NO	NO	NO	NO	NO	10/C	16	8.5	6.8	84	78	230	16	1.1	7.6	3.1	4.5	6
JAFFER	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAJA	45	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
MARAN	45	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
KUMARES/	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	28	2	0.8	78	64	240	21	2	6.2	3	3.2	33
MARIAMM	54	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	10/C	15	2.6	1.4	34	38	100	16	1.3	3	2	1	34
SUBRAMAI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
RAJAN	36	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
SOMU	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	12	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
GOVINDAR	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	9	2	0.6	76	78	180	14	0.8	4.5	2.8	1.7	36
ADAM	42	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	16	8.5	6.8	84	78	230	16	1.1	7.6	3.6	4	6
VENKATES/	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	28	2	0.8	78	64	240	21	2	6.2	3	3.2	33
RAJESWAR	38	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	7/B	6	0.8	0.4	18	21	88	13	1	7.2	4.6	2.6	28
BALAJI	48	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	11	2.2	1.6	96	84	210	14	1	4.5	2.5	2	36
ARUN KUM	24	MALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	YES	9/B	15	2.4	1.6	22	16	60	12	1	6	2.7	2.3	65
PUGAZHEN	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
CHANDRAS	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SRINIVASA	44	MALE	OP	NO	NEG	NEG	NCPF	NO	NO	NO	NO	NO	10/C	16	3.7	1.5	72	32	527	20	1.5	6.5	3.1	3.4	14
MAHESWA	38	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	7/B	6	0.8	0.4	18	21	88	13	1	7.2	4.6	2.6	28
SELVARAJ	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAMAN	54	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	10	2.4	0.8	78	84	220	14	1	4	2.5	1.5	36
BALAMURI	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SUJATHA	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
KRISHNAN	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SHADIK BA	34	MALE	OP	NO	NEG	NEG	BUDD CHI/	NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
JOTHI	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
SAMBATH	30	MALE	OP	YES	NEG	NEG		YES	NO	NO	NO	NO	8/B	7	1.2	0.2	40	36	70	14	1	5	3.5	1.5	26
VIMALA	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
GOPI	28	MALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	8/B	10	1.28	0.23	107	53	245	16	1.3	7.1	2.5	4.6	27
MURUGAN	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
JOHN BASI	44	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	11/C	17	4	2.6	88	98	230	12	1	4.5	2.7	0.7	34
SARALA	52	FEMALE	OP	NO	NEG	NEG	CRYPTOGE	NO	NO	NO	NO	NO	9/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
KRISHNAM	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
VARADARA	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SRINIVASA	75	MALE	OP	NO	22,400,00(	NEG		NO	NO	NO	NO	NO	12/C	29	2.8	2	52	35	100	13	1	4.5	3	1.5	34
BABU	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	7	1.2	0.4	55	38	316	15	1	7.8	3	4.8	30
SUNDARAL	45	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	10/C	15	10.3	6	57	28	57	13	1	6.7	3.9	2.8	22
MUNNUSA	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAMU	39	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	12/C	25	12.4	9.8	88	57	305	26	2.2	6.2	2	4.2	46
JALENDRAI	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
ALAGESAN	36	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
RAMAKRIS	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	8/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30
BABU	52	MALE	OP	YES	NEG	NEG		NO	NO	NO	NO	NO	9/B	8	1.2	0.4	55	38	316	15	1.1	7.8	3	4.8	30

PROFORMA

Name:

Age:

Sex:

Occupation:

Address:

O.P No./IP No.:

Micro ID No:

Complaints:

Past History

Personal History:

Clinical History:

H/o Previous SBP:

Treatment History:

General Examination:

Systemic Examination:

Routine investigatory findings:

Ascitic fluid Analysis:

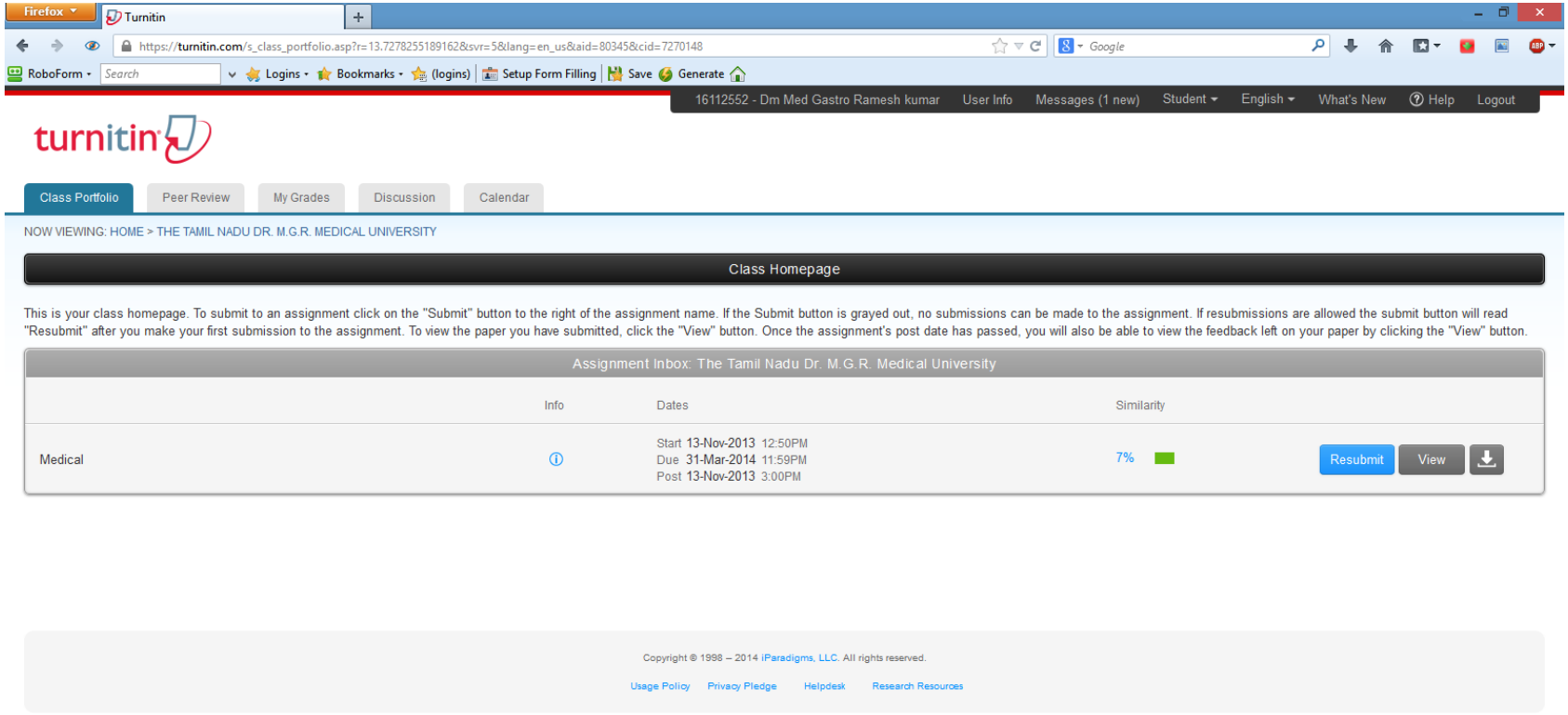
□Cell count □SAAG

□Protein □Amylase

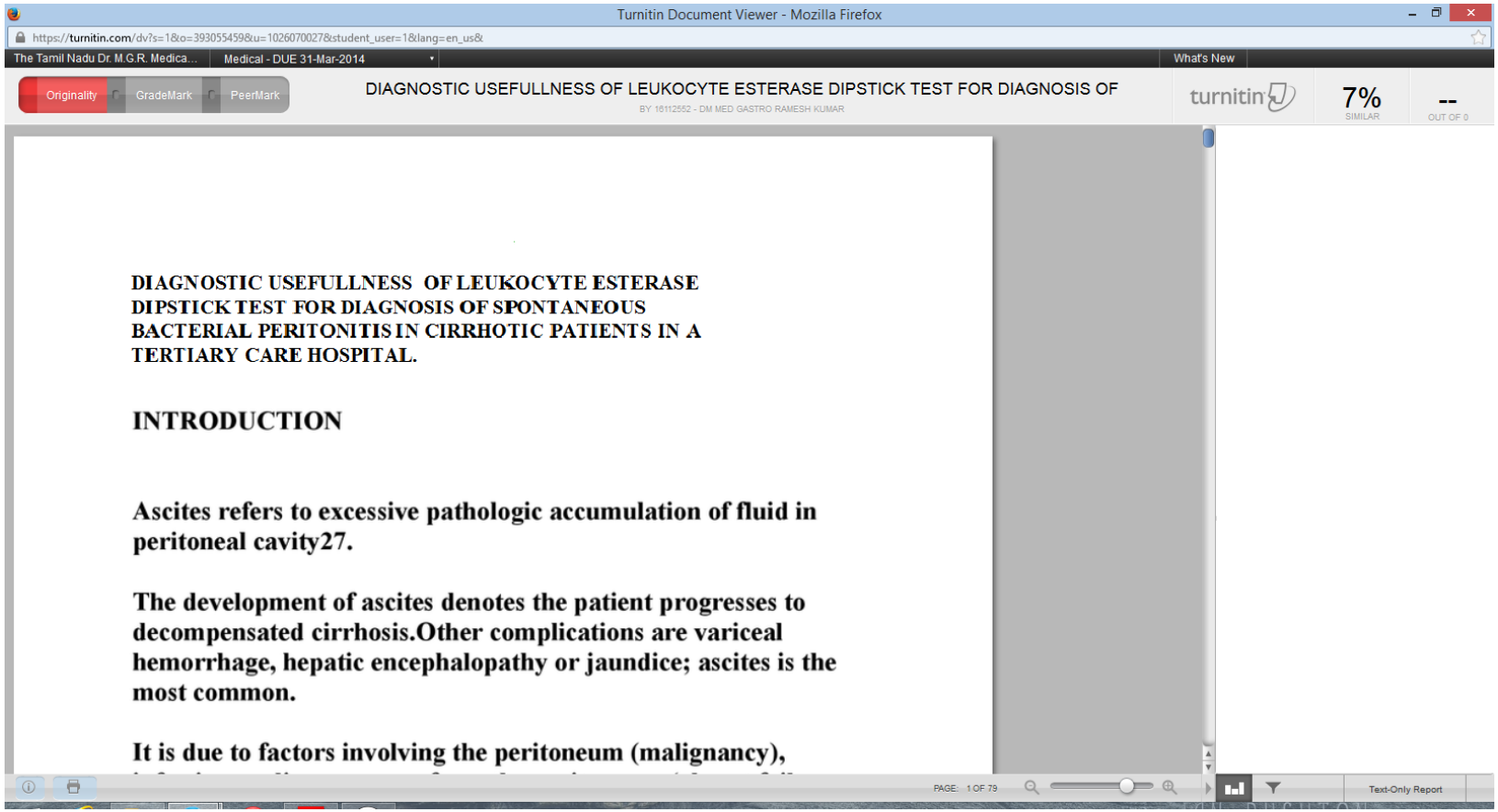
□Albumin □AFP

USG Abdomen report:

ANTI PLAGIARISM



ANTI PLAGIARISM



INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Diagnostic validity of leucocyte esterase dipstick test for diagnosis of spontaneous bacterial peritonitis in cirrhotic patients in the tertiary care hospital

Principal Investigator : Dr.T.S.Ramesh Kumar

Designation : PG in DM (Gastroenterology)

Department : Department of Medical Gastroenterology
Government Stanley Medical College,
Chennai-1

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 07.02.2013 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

15/10/13

MEMBER SECRETARY,
IEC, SMC, CHENNAI